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Express Mail No. EM218063681US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Patentee:	Charles G. COCHRANE, *et al.*	Docket No:	TSRI-147.2CO
Patent No.:	5,789,381	Group Art Unit:	1652
Issued:	August 4, 1998	Confirmation No.:	4428
For:	**PULMONARY SURFACTANT PROTEINS AND RELATED POLYPEPTIDES**	Examiner:	Patrick R. DELANEY

COVER LETTER FOR INITIAL APPLICATION FOR INTERIM EXTENSION OF PATENT TERM UNDER 35 U.S.C. §156(d)(5)

Mail Stop: **Hatch-Waxman PTE**
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

Discovery Laboratories, Inc. (hereinafter "Discovery Labs"), submits herewith an application for a first interim extension of patent term of U.S. Patent No. 5,789,381 (the "'381 patent") under 35 U.S.C. §156(d)(5). Pursuant to Discovery Labs' duty under 37 CFR §1.740(a)(13) to disclose to the Director of the U.S. Patent and Trademark Office and to the Secretary of Health and Human Services any information which is material to the determination of entitlement to the extension sought herein, Discovery Labs calls attention to its concurrent filing of applications for interim patent term extensions in U.S. Patent Nos. 5,260,273 and 5,407,914. Discovery Labs believes these concurrent filings may be material to a determination of entitlement to the interim extension sought herein.

Discovery Labs also calls attention to 35 U.S.C. §156, which states in part:

> (c) The term of a patent eligible for extension under subsection (a) shall be extended by the time equal to the regulatory review period for the approved product which period occurs after the date the patent is issued, except that-
>
> ...
>
> (4) in no event shall more than one patent be extended under subsection (e)(1) for the same regulatory review period for any product.

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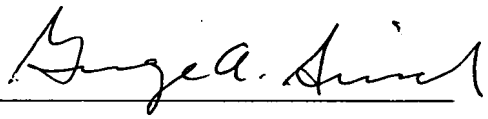
Although 35 U.S.C. §156(c)(4) prohibits extending the term of more than one patent under subsection (e)(1) for the same regulatory review period, and although U.S. Pat. Nos. 5,260,273, 5,407,914, and 5,789,381 claim a product subject to the same regulatory review, this application for interim patent

term extension is pursuant to subsection (d)(5) and not subsection (e)(1). Accordingly, Discovery Labs believes submitting applications for, and receiving, interim term extensions in more than one patent for the same regulatory review period is proper.

In the event that it is determined Discovery Labs may not obtain interim patent term extensions in more than one patent under subsection (d)(5) for the same regulatory review period, Discovery Labs provisionally elects to pursue its application for interim patent term extension in U.S. Pat. No. 5,407,914. Discovery Labs reserves its right to petition or appeal any determination that it is not entitled to obtain interim extensions under subsection (d)(5) in more than one patent.

The Patent Office is invited to contact the undersigned if any additional information is necessary.

Respectfully submitted,
DECHERT LLP



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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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INITIAL APPLICATION FOR INTERIM EXTENSION OF PATENT TERM UNDER 35 U.S.C. §156(d)(5)

Mail Stop: **Hatch-Waxman PTE**
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

Discovery Laboratories, Inc. (hereinafter "Discovery Labs"), a Delaware Corporation, hereby requests a first interim extension of patent term of U.S. Patent No. 5,789,381 (the "'381 patent") under 35 U.S.C. §156(d)(5) and in accordance with 37 CFR §1.790.

Discovery Labs represents that it has the authority to seek said first interim patent term extension pursuant to the authority granted to Discovery labs by an Appointment of Agent by Assignee to act as the sole agent of assignee Scripps Research Institute ("Scripps") to prosecute the application for extension and to handle all matters relating to the '381 patent. A copy of such Appointment of Agent by Assignee, which is being filed concurrently herewith, is included as **Exhibit A**.

Discovery Labs also includes herewith, as **Exhibit B**, documentary evidence of Discovery Labs' previous corporate names and merger with ATI Acquisition Corp., a corporation formerly known as Acute Therapeutics, Inc.

Scripps is assignee of the entire interest in the '381 patent by an assignment from the inventors, Cochrane, *et al.*, recorded at Reel 015215/Frame 0518 on Aug. 4, 1998.

Discovery Labs requests that a first interim extension of patent term of one year be granted and submits that the instant application is complete.

For convenience and ease of reference, this application is structured to correspond to the sections of 37 CFR §1.740 as directed in 37 CFR §1.790(b).

§1.740(a)(1): Complete Identification of Product

The product presently subject to regulatory review is Surfaxin® (lucinactant). Surfaxin® is under review for use in the prevention of Respiratory Distress Syndrome (RDS) in premature infants. A complete identification of Surfaxin® is provided in **Exhibit C**.

§1.740(a)(2): Identification of Federal Statute/Provision of Law

Surfaxin® is presently subject to regulatory review under 21 U.S.C. §355(c) [§505 of the Federal Food, Drug, and Cosmetic Act (FFDCA)].

§1.740(a)(3): Date on Which Product Received Permission for Commercial Marketing or Use

This section is not applicable as Surfaxin® has not yet received permission for commercial marketing under 21 U.S.C. §355(b)(1). *See also*, 37 CFR §1.790(b) explicitly noting that 37 CFR §1.740(a)(3) is not applicable to applications for interim patent term extensions.

§1.740(a)(4): Identification of Active Ingredient

Surfaxin® has the following active ingredients (as described in **Exhibit C**):

(i) sinapultide ("KL_4 peptide");
(ii) colfosceril palmitate (dipalmitoylphosphatidylcholine; "DPPC");
(iii) palmitoyloleoylphosphatidyl glycerol sodium salt ("POPG"); and
(iv) palmitic acid ("PA").

With regard to the components sinapultide and palmitoyloleoylphosphatidyl glycerol, sodium salt, Applicant states that they have not been previously approved for commercial marketing or use under the Federal Food, Drug, and Cosmetic Act, the Public Health Service Act, or the Virus-Serum-Toxin Act.

With regard to component colfosceril palmitate (DPPC), Applicant states that DPPC was previously approved for commercial marketing on August 2, 1990 under the FFDCA (NDA #020044; Exosurf), for neonatal respiratory distress syndrome. DPPC is also used in the formulation of Survanta. Beractant (and not DPPC) is the active ingredient of Survanta and was approved for commercial marketing on July 1, 1991, under the FFDCA (NDA #020032; Survanta), for neonatal respiratory distress syndrome. DPPC is also used in the formulation of Curosurf. Poractant alfa (and not DPPC) is the active ingredient of Curosurf and was approved for commercial marketing on Nov. 18, 1999, under the FFDCA (NDA #020744; Curosurf), for neonatal respiratory distress syndrome.

With regard to component palmitic acid (PA), Applicant states that PA is used in the formulation of Survanta. Beractant (and not PA) is the active ingredient of Survanta and was approved for commercial marketing on July 1, 1991, under the FFDCA (NDA #020032; Survanta), for neonatal respiratory distress syndrome.

The previous approval of DPPC under the FFDCA, and the use of DPPC and PA in approved products as non-active ingredients, does not preclude a patent term extension to U.S. Patent No. 5,789,381 based on the product, which comprises a combination of sinapultide, DPPC, POPG, and PA. *See, e.g. The Arnold Partnership v. Dudas*, 362 F.3d 1338, 1341 (Fed. Cir. 2004) ("To extend the term of a patent claiming a composition comprising A and B, either A or B must not have been previously marketed. In other words, at least one of the claimed active ingredients must be new to the marketplace as a drug product.").

§1.740(a)(5): Time Period for Submitting Application

As noted in 37 CFR §1.790(b), this section is not applicable to initial applications for interim patent term extensions. However, it is noted that this initial application for interim patent term extension is being timely submitted within the period specified by 37 CFR §1.790(a) for such initial applications of May 17, 2009 to Nov 2, 2009, which is the period beginning six months and ending fifteen days before the term of the '381 patent would otherwise expire.

§1.740(a)(6): Identification of Patent

The patent for which patent term extension is being sought is U.S. Patent No. 5,789,381, which issued on August 4, 1998 to inventors Charles G. Cochrane and Susan D. Revak and which is entitled "Pulmonary Surfactant Proteins and Related Polypeptides." The term of U.S. Patent No. 5,789,381 will expire, unless extended, on Nov. 17, 2009.

§1.740(a)(7): Copy of Patent

For the convenience of the Office, a copy of the '381 patent is included herewith as **Exhibit D**.

§1.740(a)(8): Copies of Disclaimers, etc.

The fourth and eighth year maintenance fees for the '381 patent have been paid. Copies of the maintenance fee statements (from the U.S. Patent and Trademark Office website) verifying the payments are included as **Exhibit E**. The twelfth year maintenance fee window is currently open.

A copy of the single Certificate of Correction issued by the U.S. Patent and Trademark Office for the '381 patent is attached as **Exhibit F**.

No reexamination certificates have issued for the '381 patent.

Copies of the two terminal disclaimers filed in the '381 patent are attached as **Exhibit G**.

§1.740(a)(9): Claims Covering the Product or Method of Using the Product

The '381 patent contains six claims. Claims 1–3 of U.S. Patent No. 5,789,381 read on the product for which approval is sought, and claims 4–6 read on a method of using the product for which approval is sought.

Independent claim 1 recites:

> A pulmonary surfactant comprising one or more pharmaceutically acceptable phospholipids admixed with a polypeptide having an amino acid residue sequence represented by the formula KLLLLKLLLLKLLLLKLLLLK (SEQ. ID NO: 1), said polypeptide, thereby forming a pulmonary surfactant having a surfactant activity greater than the surfactant activity of the phospholipid alone, said phospholipid being present in the range of about 50-100 weight percent, in a polypeptide:phospholipid weight ratio in the range of about 1:7 to about 1:1,000.

As required by 37 CFR §1.740(a)(9)(i), at least independent claim 1 reads on the product for which approval is being sought. That product includes one or more pharmaceutically acceptable phospholipids (DPPC and/or POPG) and a peptide (KL_4 peptide) represented by SEQ ID NO: 1. The phospholipid(s) are present within the claimed range of 50-100 weight percent, in a polypeptide:phospholipid weight ratio in the range of about 1:7 to about 1:1,000. Exact phospholipid weight percentage and polypeptide:phospholipid weight ratio are not disclosed as being proprietary. Furthermore, said KL_4 peptide, when admixed with a pharmaceutically acceptable phospholipid (DPPC and/or POPG), forms a pulmonary surfactant having a surfactant activity greater than the surfactant activity of the phospholipid alone.

Independent claim 4 recites:

> A method of treating respiratory distress syndrome comprising administering a therapeutically effective amount of a pulmonary surfactant, said surfactant comprising one or more pharmaceutically acceptable phospholipids admixed with a polypeptide having an amino acid residue sequence represented by the formula KLLLLKLLLLKLLLLKLLLLK (SEQ. ID NO: 1), said polypeptide, when admixed with a pharmaceutically acceptable phospholipid, forming a pulmonary surfactant having a surfactant activity greater than the surfactant activity of the

> phospholipid alone, said phospholipid being present in the range of about 50-100 weight percent, in a polypeptide:phospholipid weight ratio in the range of about 1:7 to about 1:1,000, or in an amount such that it may be administered in a range of about 50 mg/kg to about 500 mg/kg per dose.

As required by 37 CFR §1.740(a)(9)(i), at least independent claim 4 reads on a method of using the product for which approval is sought. Approval of the product is being sought for the prevention of respiratory distress syndrome in premature infants. The prophylactic therapy comprises administering a therapeutically effective amount of a synthetic pulmonary surfactant comprising one or more pharmaceutically acceptable phospholipids (DPPC and/or POPG) and a polypeptide (KL_4 peptide) represented by SEQ ID NO: 1. The phospholipid(s) are present within the claimed range of 50-100 weight percent, in a polypeptide:phospholipid weight ratio in the range of about 1:7 to about 1:1,000. The product is administered in a dosage range of about 50 mg/kg to about 500 mg/kg per dose. Exact phospholipid weight percentage, polypeptide:phospholipid weight ratio, and dose are not disclosed as being proprietary. Furthermore, said KL_4 peptide, when admixed with a pharmaceutically acceptable phospholipid (DPPC and/or POPG), forms a pulmonary surfactant having a surfactant activity greater than the surfactant activity of the phospholipid alone.

§1.740(a)(10(i)): Relevant Dates

The relevant dates and information required by 35 U.S.C. §156(g) and 37 CFR §1.740(a)(10)(i) are as follows:

Investigational New Drug Application ("IND") No. 40,287 was filed with the FDA by July 31, 1992 and became effective on Sept 5, 1992 (37 CFR §1.740(a)(10)(i)(A)).

New Drug Application ("NDA") No. 21-746 was submitted by April 13, 2004 and accepted on June 12, 2004 (37 CFR §1.740(a)(10)(i)(B)).

The NDA is under review and has not yet been approved (37 CFR §1.740(a)(10)(i)(C)).

§1.740(a)(11): Brief Description of Significant Activities

A brief description of the significant activities undertaken during the regulatory review period by Discovery Labs, including under its previous name, Acute Therapeutics, Inc ("ATI") and its predecessor, Scripps, with the FDA regarding Surfaxin® is presented in the chronology attached hereto as **Exhibit H**. The activities began with the date IND No. 40,287 was submitted to the FDA by Scripps followed by the date NDA No. 21-746 was submitted to the FDA by Discovery Labs and continue until the date of the present application.

In the chronology, Scripps is shown to have performed the activities until the sponsorship of IND No. 40,287 was transferred to ATI on or about Dec. 4, 1996. ATI later changed its company name to Discovery Laboratories, Inc. and notified the FDA of this change on or about June 29, 1998 (see **Exhibit I**). Discovery Labs filed NDA No. 21-746 which is currently under review by the FDA.

The following chronologies, also attached as **Exhibits H** and **I**, provide additional details of the significant activities undertaken during the regulatory review period for IND No. 40,287 and NDA No. 21-746.

SURFAXIN® US IND #40,287 Brief Description of Significant Events

Date	*Type of Document*	*Description*
7/31/1992	Original Submission	Scripps Institute (Sponsor Charles G. Cochrane, MD) filed IND for SURFAXIN with the FDA under Section 505(i) of the Federal Food, Drug and Cosmetic Act and 21 CFR §312.
8/6/1992		The date of receipt of IND by the FDA.
8/11/1992	FDA Letter	Acknowledgement of receipt of IND Application.
8/25/1992	Scripps Letter	Suggested Modifications in the Clinical Protocol Pursuant to Discussions with Dr. Himmel at FDA.
8/31/1992	Scripps Letter	Letter to Dr. Himmel from Dr. Cochrane adding information to provide supporting data for dosage form.
9/1/1992	FDA Letter	Additional comments of safety and consent form from Dr. Himmel.
9/3/1992	Scripps Letter	Resulting changes per Dr. Himmel's suggestions in letter dated 9/1/92.
9/10/1992	Scripps Letter	Revised Patient Consent Form for Protocol.
9/11/1992	FDA TELEFAX	Fax from FDA regarding the Patient Consent Forms.
9/25/1992	Scripps Letter	Revised Patient Consent Forms with revisions according to the requested FDA guidelines in your FAX of 9/11/92.
1/8/1993	Scripps Letter	Final Consent Forms from the 3 University of California campuses.
3/17/1003	Scripps Letter	Expansion of weight range request.
3/19/1993	Scripps Letter	Addendum for drug dose.
4/19/1993	FDA Letter	Comments on preclinical studies re 1/8/93 amendment.
5/19/1993	Scripps Letter	Reply to 4/19/93 FDA letter re: IND # 40,287.

Date	*Type of Document*	*Description*
6/30/1993	Protocol Amendment: Change in Protocol Information Amendment: Chemistry/Microbiology Information Amendment: Pharmacology/Toxicology	Amendment to CP 1 protocol , (CP 1 to be referred to as CP 2) Introductory Statement, Investigator's Brochure, Protocol, CMC, Pharm/Tox, and Previous Human Experience. Minor changes to investigator's brochure. Protocol CP-2 (high dose). CMC changes to higher dose.
8/4/1993	Scripps Letter	Minor protocol change to Amendment # 001 (6/30/93)
9/7/1993	FDA Letter	Comments on Amendment 6/30/93. FDA is continuing to review this amendment, however, they see no objectionable data to increasing the dose. Recommends that subjects are randomized to the two doses so that dose response data can be obtained.
9/21/1993	Protocol Amendment: New Investigator	Added four (4) new investigators to CP2 Protocol. Attachments: relevant form FDA 1572s and CVs; Copy of ICF from each new sites
10/27/1993	Scripps Letter	Proposed changes to Amendment 001.
10/29/1993	Scripps Letter	Scripps grants R.W Johnson Pharmaceutical Research Institute (RWJPRI) permission to send direct communications to Scripps IND #40,287.
11/2/1993	Annual Report	1993 Annual Report
11/5/1993	Scripps Letter	Submission of pre-clinical protocol TSRI # 94-1 for FDA review.
11/18/1993	FDA TELEFAX	Comments from complete medical review from 10/27/1993 amendment
11/18/1993	FDA Letter	Review of Scripps Letter dated 10/27/93.
11/19/1993	FDA Letter	Comments from FDA on submission dated 11/5/93.
11/19/1993	Scripps Letter	Letter to confirm receipt of changes to protocol for newborn rabbit studies.
12/29/1993	Scripps Letter	Himmel's request for information on high frequency ventilation in infants with RDS receiving surfactant.
9/21/1994	Annual Report	1994 Annual Report.
4/13/1995	Scripps Letter	Request End of Phase II Conference.
5/19/1995	Scripps Letter	Partial transfer of sponsor responsibilities from Scripps to Beardsworth Consulting Group.
5/19/1995	General Correspondence: Response to FDA Request for Information	Response to FDA Request for Information. Proposed agenda for End of Phase II conference; Letter of Authorization to cross-reference PRI IND 46,164; Clinical Data Summary from CP1 and CP2; Listing of proposed Phase 2 Clinical Sites; CP-3 Protocol (Draft #4, 5/17/95); Brief summary of CP4; Summary of CM&C changes; Summary of Pharmacology/toxicology.
7/20/1995	Scripps Letter	Information obtained from the Orphan Drug application.
8/23/1995	General Correspondence: Pre-Meeting Package: End of Phase II Meeting	Revised Clinical Protocols (KL4-IRDS-001and -002); Revised Table 10 of Clinical Data Summary from Phase I/II; Updated Toxicology Tables and Summary of Planned Multiple Dose Rat Study; Summary of Planned Drug Metabolism Studies; Summary of Planned Preclinical Pharmacology Studies; Updated Proposed Agenda; List of attendees for 09/11/1995 End of Phase II RDS Meeting.
8/24/1995	Information Amendment: Chemistry/Microbiology Information Amendment: Clinical Information Amendment: Pharmacology/Toxicology	New Investigator's Brochure; Cross-reference to CM&C in 46,164. Pharm/Tox- overview, summary tables, summaries of pre-clinical studies filed to 46,164; Filing of TSRI 94-1; Updated human experience.
10/10/1995	Scripps Letter	Summary of 9/11/95 End of Phase II Meeting.

Date	*Type of Document*	*Description*
10/20/1995	Annual Report	1995 Annual Report.
10/31/1996	Annual Report	1996 Annual Report.
12/4/1996	General Correspondence: Transfer of Sponsorship	Transfer of Sponsorship from Scripps to Acute Therapeutics, Inc. (ATI).
12/5/1996	General Correspondence: Acceptance of Ownership	Acceptance of Ownership and Responsibility by ATI.
12/13/1996	FDA TELEFAX	Meeting minutes from the FDA. Meeting was held to discuss the proposed study design for Protocol KL4-IRDS-001 as well as other issues pertinent to an NDA Submission.
12/18/1996	FDA Letter	Acceptance of Transfer of 40,287 to Acute Therapeutics, Inc.
3/25/1997	Information Amendment: Pharmacology/Toxicology	Pharm/Tox: Includes 3 final reports ; Mass Balance Determination of 3H-KL4 Surf. In Rats Following A Single Intratracheal Dose of 3H-KL4 Surf; Acute Intratracheal Safety of RWJ-45652-021 in Crl:CDBR, VAF/Plus Rats; 7 Day Intratracheal Toxicity Study of RWJ 45652-021.
3/27/1997	FDA TELEFAX	FDA's 3/10/97 meeting minutes.
4/17/1997	Information Amendment: Chemistry/Microbiology	Revised CMC section of IND 40,287 I: Introduction, II: Drug Substance, III: Other active ingredients, IV: Drug Product, V: Environmental Assessment.
6/6/1997	Information Amendment: Clinical	Curriculum Vitae of new Medical Monitor for Discovery Laboratories, Inc.
7/3/1997	Information Amendment: Chemistry/Microbiology	Two-month Stability Reports for SURF-0001 & SURF- 0002.
9/30/1997	Annual Report	1997 Annual Safety Report. New Information Included: Preclinical Study Information and Manufacturing and/or Microbiological Changes.
1/14/1998	Information Amendment: Chemistry/Microbiology	Amended CMC section to correct an inadvertent error in the container-closure system information for the finished drug substance.
3/31/1998	Information Amendment: Chemistry/Microbiology	PCD Report (PCD-98-002) on the validity of the Dynamic Surface Tension Assay. Revised Surfaxin stability protocol.
6/29/1998	General Correspondence: Change in Company Name	Change in company name from Acute Therapeutics Inc. to Discovery Laboratories Inc.
9/8/1998	Information Amendment: Clinical	Full clinical report for protocols CP-1 and CP-2.
10/30/1998	Annual Report	Annual Safety Report for the period August 1, 1997 to July 31, 1998.
6/4/1999	DSCO Telefax	Follow-up to 6/4/99 telecon regarding FDA comments on proposed phase 3 equivalency trial in RDS. Fax provided protocol concept sheet and Position Paper
7/2/1999	Information Amendment: Clinical	Protocol concept sheet for Surfaxin/Survanta equivalency trial and a supporting position paper.
8/12/1999	General Correspondence: Request for End-of-Phase 2 Meeting	End of Phase 2 meeting agenda, discussion items, introduction, preclinical overview, clinical overview and CMC overview.
8/19/1999	FDA TELEFAX	Comments on proposed Phase 3 equivalency trial in RDS.
8/19/1999	FDA Letter	Comments regarding the proposed Phase 3 protocol for RDS.
9/3/1999	DSCO Telefax	Discussion items for the September 8, 1999 Phase 3 IRDS trial teleconference
9/10/1999	DSCO Telefax	Proposed equivalency prevention study comparing Surfaxin and Survanta; Discovery's minutes from the September 8, 1999 teleconference
9/10/1999	DSCO Telefax	1999 IRDS teleconference

Date	*Type of Document*	*Description*
9/27/1999	FDA TELEFAX	List of FDA attendees for the 10/14/99 End-of-Phase 2 meeting.
11/3/1999	FDA TELEFAX	FDA Comments on End-of-Phase 2 Meeting Package
11/3/1999	Annual Report	Annual Safety Report for the period August 1, 1998 to July 31, 1999.
11/12/1999	General Correspondence: Response to FDA	10/14/99 end-of-phase 2 Discovery meeting minutes.
12/9/1999	FDA Correspondence	FDA comments regarding Discovery's September 10, 1999 meeting minutes from September 9, 1999 telecon.
1/6/2000	FDA TELEFAX	FDA Comments official comments on Discovery meeting minutes from Discovery/FDA teleconference date 9/9/99.
1/12/2000	FDA TELEFAX	FDA agenda for 1/14/00 meeting.
1/12/2000	FDA TELEFAX	Comments on proposed Phase 3 RDS protocol concept.
1/12/2000	DSCO Telefax	Proposed Phase 3 IRDS protocol concept.
1/24/2000	FDA TELEFAX	FDA provided Discovery's meeting minutes revised which were now the agency's official minutes. In addition, they provided several CMC comments.
2/3/2000	General Correspondence	Copy of Discovery meeting minutes from January 14, 2000 RDS Meeting at the FDA. Copy of Statistical Presentation given by Dr. Tsai
2/8/2000	General Correspondence: RDS Phase 3 Clinical Trial Proposal	Revised RDS protocol concept sheet; Study design rationale paper.
3/1/2000	FDA TELEFAX	FDA comments regarding the revised RDS protocol concept sheet for a Surfaxin-Survanta superiority trial (Fax cover page dated 02/28/2000, Fax banner 03/01/2000).
3/1/2000	FDA TELEFAX	FDA meeting minutes from January 14, 2000 meeting regarding the proposed RDS equivalency prevention study comparing Surfaxin with Survanta.
3/3/2000	DSCO Telefax	Discovery's response to the agency's comments regarding the phase 3 protocol concept sheet and rationale.
3/8/2000	DSCO Telefax	Discovery's response to the agency's request for information and Discovery's meeting minutes from the 3/6/00 teleconference regarding the proposed phase 3 RDS protocol concept sheet and rationale.
3/13/2000	General Correspondence: Response to FDA General Correspondence: Development/Meeting Min	Response to the Agency's comments in the 3/1/00 faxed letter; a copy of the faxed letter; the Discovery meeting minutes from the 3/6/00 teleconference regarding phase 3 RDS concept sheet submitted 2/8/2000; and Discovery's response to the Agency's request for information.
3/28/2000	DSCO Telefax	Discovery alternate RDS protocol proposal.
3/31/2000	DSCO Telefax	Discovery meeting minutes from 3/29/00 teleconference regarding the composite primary endpoints proposed in the phase 3 RDS protocol concept sheet submitted 02/08/2000 serial no 049.
4/4/2000	General Correspondence: Development/Meeting Minutes	Discovery's alternative RDS non-inferiority proposal dated 03/28/2000; Discovery's meeting minutes from the 3/29/00 teleconference regarding composite primary endpoints proposed in the Phase 3 RDS protocol concept sheet.
4/7/2000	DSCO Telefax	Supplement to noninferiorty Surfaxin vs Survanta RDS protocol proposal.
4/14/2000	FDA TELEFAX	Agency meeting minutes from the 3/6/00 teleconference regarding DCSO responses to agency comments on submitted phase 3 RDS protocol concept sheet.

Date	*Type of Document*	*Description*
4/18/2000	FDA TELEFAX	Agency concerns about the current proposal for non-inferiority trial of prophylaxis Surfaxin vs rescue Survanta in the treatment of RDS
5/23/2000	FDA TELEFAX	April 14, 2000 Teleconference minutes regarding the agency concerns for the proposed non-inferiority study
5/26/2000	Information Amendment: Chemistry/Microbiology	CMC Amendment which includes changes in support of the MAS and IRDS indications.
5/26/2000	General Correspondence Information Amendment: Pharmacology/Toxicology	Minutes from April 14, 2000 teleconference; Pharm/tox study.
8/15/2000	Information Amendment: Clinical	Curriculum Vitae of Robert Segal, M.D., new Medical Monitor for Discovery Laboratories, Inc.
8/16/2000	General Correspondence	August 9, 2000 Teleconference meeting minutes regarding performing a RDS placebo controlled trial.
8/30/2000	FDA TELEFAX	Agency telecon meeting minutes for the 8/9/00 teleconference regarding the proposed RDS trial..
10/12/2000	FDA TELEFAX	Revised version of agency meeting minutes from 8/9/00 teleconference regarding the proposed RDS study..
11/13/2000	Protocol Amendment: New Protocol	Protocol KL4-IRDS-04 dated 11/10/2000; Discovery minutes from 8/9/00 teleconference regarding the feasibility of conducting a placebo-controlled RDS trial in Latin America.
11/27/2000	FDA TELEFAX	Letter from agency regarding ethical and data applicability issues with the regard to the proposed phase 3 RDS study.(Received via fax).
11/27/2000	FDA Letter	Letter from agency regarding ethical and data applicability issues with the regard to the proposed phase 3 RDS study. (original FDA letter received 12/4/2000)
12/14/2000	DSCO Telefax	Justification as to why the planned phase 3 RDS study is ethical and an appropriate trial design to support an U.S. marketing application.
1/11/2001	FDA TELEFAX	Agency meeting minutes from 12/18/00 teleconference regarding Discovery's position with respect to the ethicality and data applicability of the proposed phase 3 RDS trial design
1/17/2001	Annual Report	2000 Annual Safety Report for the period August 1, 1999 to July 31, 2000.
1/18/2001	Protocol Amendment: Change in Protocol General Correspondence: Response to FDA	Response to Agency from 12/18/00 telecon; Copy of Agency's 12/18/00 meeting minutes; Copy of DSCO 12/18/00 meeting minutes regarding ethicality/approvability of proposed phase 3 RDS; Change doc for protocol KL4-IRDS-04 Amend 1; Protocol KL4-IRDS-04 Amend 1; Training syllabus; Synopsis of proposed European Phase 3 RDS study; A copy of Discovery's meeting minutes from the 12/11/00 meeting with the EMEA.
1/26/2001	Information Amendment: Chemistry/Microbiology	CMC Amendment which includes information in support of the MAS and IRDS indications.
2/20/2001	Information Amendment: Clinical	Updated Investigator Brochure dated February 6, 2001.
2/22/2001	DSCO Telefax	Fax to Dr. Myer, follow up to telecon on 2/22/01. Copy of letter sent to Secretary Thompson by the Public Citizen, and notations on the fact that Dr. Sidney Wolfe and cast had a copy of Birenbaums presentation.
2/26/2001	Protocol Amendment: New Protocol Information Amendment: Clinical	Protocol KL4-IRDS-02; KL4-IRDS-02 sample CRF; KL4-IRDS-02 sample ICF; DSCO's 12/14/00 facsimile transmission; KL4-IRDS-04 CRF; KL4- IRDS-04 sample ICF.

Date	*Type of Document*	*Description*
3/8/2001	DSCO Telefax	Pre-meeting package for 3-12-01 meeting with the FDA: Provided 1. Proposed Discovery meeting attendees; 2. Proposed agenda; 3. Introduction and background; and 4. An alternate RDS trial design
3/8/2001	General Correspondence: March 12, 2001 Pre-Meeting Package	Proposed Discovery meeting attendees; Proposed meeting agenda; Introduction and background; An alternate trial design.
3/9/2001	FDA TELEFAX	FDA telefax providing list of attendees for 3-12-01 meeting; a proposed agenda; and comments regarding the proposed placebo-controlled trial in Latin America.
3/9/2001	FDA Letter	Formal letter from agency detailing comments regarding KL4-IRDS-04, placebo-controlled RDS study planned for Latin America
3/14/2001	DSCO Telefax	Follow-up from 3-12-01 meeting. Discover provided a revised trial design.
3/29/2001	FDA TELEFAX	Comments on prophylaxis superiority trial with Surfaxin or Survanta rescue.
3/19/2001	FDA TELEFAX	Response to alternate proposal
3/26/2001	DSCO Telefax	Follow-up to the 3-22-01 teleconference with the FDA. Provided: a new RDS trial design.
5/21/2001	General Correspondence: Teleconference/Meeting Minutes Protocol Amendment: New Protocol	Protocol KL4-IRDS-06, Discovery's Meeting Minutes from 3/12/01 regarding phase 3 RDS; 3/22/01 regarding phase 3 RDS; 3/29/01 regarding proposed alternative trial; 4/4/01 regarding proposed alternative trial.
5/22/2001	FDA TELEFAX	FDA Meeting Minutes from 4/4/01 regarding proposed alternative trial.
5/22/2001	FDA TELEFAX	FDA Meeting Minutes from 3/29/01 regarding proposed alternative trial.
5/22/2001	FDA TELEFAX	FDA Meeting Minutes from 3/22/01 regarding phase 3 RDS
5/22/2001	FDA TELEFAX	FDA Meeting Minutes from 3/12/01 regarding phase 3 RDS
6/4/2001	General Correspondence	Discovery Hardcopy's of facsimile transmissions: 3/14/ 2001 revised trial design based on agency feedback during the March 12, 2001 meeting, 3/26/01 revised trial design, 3/30/01 revised trial design and 4/4/01 requested a copy of the agency's meeting mins from the 3/12/01 meeting and the telecon held on 3/22/01 & 3/29/01.
8/16/2001	FDA Telefax	Clinical Comments for protocols KL4-IRDS-02 and KL4-IRDS-06
8/29/2001	Protocol Amendment: New Investigator	Added three (3) new Investigators for protocol KL4-IRDS-02. Attachments: relevant form FDA 1572s and CVs.
8/29/2001	Protocol Amendment: New Investigator	Added one (1) new Investigator for protocol KL4-IRDS-06; Attachments: relevant form FDA 1572 and CV.
9/5/2001	DSCO Telefax	Submitted IND Safety Report - 7 Day Alert (Initial).
9/5/2001	IND Safety Report: 7-Day Alert (Initial)	Submitted IND Safety Report - 7 Day Alert (Initial).
9/12/2001	DSCO Telefax	Submitted IND Safety Report - 7-Day Alert (Initial).
9/13/2001	DSCO Telefax	Submitted IND Safety Report - 7-Day Alert (Initial).
9/13/2001	IND Safety Report: 7-Day Alert (Initial)	Submitted IND Safety Report - 7-Day Alert (Initial).
9/18/2001	DSCO Telefax	Submitted IND Safety Report - 7-Day Alert (Initial).
9/18/2001	IND Safety Report: 7-Day Alert (Initial)	Submitted IND Safety Report - 7-Day Alert (Initial).

Date	*Type of Document*	*Description*
9/26/2001	FDA Letter	Clinical and Statistical comments from the FDA on Protocol KL4-IRDS-02/06
9/26/2001	FDA Telefax	Clinical Comments from FDA on Protocol # KL4-IRDS-02 and 06
9/26/2001	DSCO Telefax	Submitted IND Safety Report - 7-Day Alert (Initial).
9/27/2001	IND Safety Report: 15-Day Alert (Initial)	Submitted IND Safety Report - 15 Day Alert (Initial).
9/27/2001	IND Safety Report: 15-Day Alert (Initial)	Submitted IND Safety Report - 15-Day Alert (Initial).
10/1/2001	DSCO Telefax	Submitted IND Safety Report - 15-Day Alert (Initial).
10/1/2001	IND Safety Report: 15-Day Alert (Initial)	Submitted IND Safety Report - 15-Day Alert (Initial)..
10/4/2001	IND Safety Report: 7-Day Alert (Initial)	Submitted IND Safety Report - 7-Day Alert (Initial).
10/19/2001	Protocol Amendment: Change in Protocol	Change Document for Protocol KL4-IRDS-06 Amendment 1; Protocol KL4-IRDS-06 Amendment 1; DSCO's response to Agency's comments regarding KL4-IRDS-06; Agency's comments regarding protocol KL4-IRDS-06.
10/31/2001	DSCO Telefax	Submitted IND Safety Report - 7 Day Alert (Initial).
11/14/2001	IND Safety Report: 7-Day Alert (Initial)	Submitted IND Safety Report - 7 Day Alert (Initial).
11/20/2001	DSCO Telefax	Submitted IND Safety Report - 7 Day Alert.
11/20/2001	DSCO Telefax	Submitted IND Safety Report: 7-Day Alert (Initial).
11/20/2001	Protocol Amendment: New Investigator	Added five (5) new Investigators for Protocol KL4-IRDS-02; Attachments: relevant form FDA 1572s and CVs.
11/20/2001	Protocol Amendment: New Investigator	Added four (4) new Investigators for protocol KL4-IRDS-06; Attachments: relevant form FDA 1572s and CVs.
11/20/2001	IND Safety Report: 7-Day Alert (Initial)	Submitted IND Safety Report - 7 Day Alert.
1/7/2002	Protocol Amendment: Change in Protocol Information Amendment: Clinical	Change document for protocol KL4-IRDS-06 Amendment 2; Protocol KL4-IRDS-06 Amendment 2; Change document for KL4-IRDS-06 Amendment 2 CRF ; CRF for KL4-IRDS-06 Amendment 2.
1/7/2002	Protocol Amendment: New Investigator	Added five (5) new investigators protocol KL4-IRDS-02; Attachments: relevant form FDA 1572s and CVs.
1/20/2002	DSCO Telefax	Submitted IND Safety Report - 7 Day Alert (Initial).
1/22/2002	Protocol Amendment: Change in Protocol Information Amendment: Clinical	DSCO's response to FDA comments on Protocol KL4-IRDS-02 -US; Agency's comments on Protocol KL4- IRDS-02-US; Change documents for protocol KL4-IRDS-02 -US Amendment 1; Protocol KL4-IRDS-02 -US Amendment 1; Change documents for Protocol KL4-IRDS-02 –US Amendment 1 CRF; Protocol KL4-IRDS-02 -US Amendment 1 CRF; Change document for ICF; Sample ICF.
1/22/2002	Information Amendment: Clinical	Changed document for sample informed consent, and revised sample consent for protocol KL4-IRDS-06.
1/24/2002	DSCO Telefax	Submitted IND Safety Report - 7 Day Alert (Initial).
1/24/2002	IND Safety Report: 7-Day Alert (Initial)	Submitted IND Safety Report - 7 Day Alert (Initial).
1/25/2002	DSCO Telefax	Submitted IND Safety Report - 15 Day Alert (Initial).
1/25/2002	IND Safety Report: 15-Day Alert (Initial)	Submitted IND Safety Report - 15 Day Alert (Initial).
2/7/2002	DSCO Telefax	Submitted IND Safety Report - 7 Day Alert (Initial).
2/18/2002	DSCO Telefax	Submitted IND Safety Report - 7 Day Alert (Initial).

Date	*Type of Document*	*Description*
2/18/2002	IND Safety Report: 7-Day Alert (Initial)	Submitted IND Safety Report - 7 Day Alert (Initial).
2/26/2002	IND Safety Report: 7-Day Alert (Initial)	Submitted IND Safety Report - 7 Day Alert (Initial).
2/26/2002	IND Safety Report: 7-Day Alert (Initial)	Submitted IND Safety Report - 7 Day Alert (Initial).
2/27/2002	DSCO Telefax	List of DSCO personnel who will be attending the 3/4/02 teleconference.
2/27/2002	Annual Report	2001 Annual Safety Report covering the period from 8/1/2000 to 7/31/2001.
3/5/2002	Information Amendment: Clinical	Clinical Trial Data and Safety Monitoring Board Standard Operating Procedure Manual.
3/6/2002	FDA Fax	FDA Statistical and clinical comments for submission 103 dated 1/7/02
3/6/2002	Information Amendment: Clinical	Deleted three (3) principal investigators from protocol KL4-IRDS-02
3/19/2002	DSCO Telefax	Submitted IND Safety Report - 7 Day Alert (Initial).
3/22/2002	FDA Fax	Request for info.
4/11/2002	IND Safety Report: 7-Day Alert (Initial) Information Amendment: Clinical	Submitted IND Safety Report - 7 Day Alert (Initial).
4/12/2002	DSCO Telefax	Submitted IND Safety Report - 7 Day Alert (Initial).
4/17/2002	DSCO Telefax	Submitted IND Safety Report - 7 Day Alert (Initial).
4/24/2002	General Correspondence: Response to FDA Request for Information	Response to Request for Information for the sites, includes documents: 3/22/02 FDA faxed information request; and DSCO response.
4/28/2002	DSCO Telefax	Submitted IND Safety Report - 7 Day Alert (Initial).
4/29/2002	General Correspondence: Meeting Minutes General Correspondence: Response to FDA	DSCO's meeting minutes from 3/4/02 teleconference; Agency's comments (3/6/02); DSCO's response to comments; Final Statistical Analysis Plan for IRDS-06, and a draft SOP manual for IRDS-06 adjudication committee.
4/29/2002	IND Safety Report: 7-Day Alert (Initial) Information Amendment: Clinical	Submitted IND Safety Report - 7 Day Alert (Initial). Amendment to the IB in the form of an Investigator Letter.
4/29/2002	IND Safety Report: 7-Day Alert (Initial) Information Amendment: Clinical	Submitted IND Safety Report - 7 Day Alert (Initial). Amendment to the IB in the form of an Investigator Letter.
4/30/2002	IND Safety Report: 7-Day Alert (Initial) Information Amendment: Clinical	Submitted IND Safety Report - 7 Day Alert (Initial). Amendment to the IB in the form of an Investigator Letter.
5/6/2002	Protocol Amendment: New Investigator Protocol Amendment: Change in Investigator	Added new investigators to protocol KL4-IRDS-02; Attachments: relevant form FDA 1572s and CVs.
5/7/2002	IND Safety Report: 7-Day Alert (Initial) Information Amendment: Clinical	Submitted IND Safety Report - 7 Day Alert (Initial). Amendment to the IB in the form of an Investigator Letter.

Date	*Type of Document*	*Description*
5/7/2002	Protocol Amendment: New Investigator	Added new Investigators to protocol KL4-IRDS-02;Attachments: relevant form FDA 1572s and CVs.
5/17/2002	General Correspondence	Correction to Serial No. 123 sent on 5/6/02 which had the incorrect protocol title. This submission contains the correct protocol title.
5/24/2002	DSCO Fax	Submitted IND Safety Report - 15 Day Alert (Initial).
5/24/2002	IND Safety Report: 15-Day Alert (Initial)	Submitted IND Safety Report - 15 Day Alert (Initial).
5/28/2002	DSCO Telefax	Submitted IND Safety Report - 7 Day Alert (Initial).
6/3/2002	Information Amendment: Clinical	Amendment to the IB in the form of an Investigator Letter dated May 28, 2002; The SAE reporting.
6/4/2002	IND Safety Report: 7-Day Alert (Initial) Information Amendment: Clinical	Submitted IND Safety Report-7 Day Alert (Initial). Amendment to the IB in the form of an Investigator Letter.
6/12/2002	Protocol Amendment: New Investigator Protocol Amendment: Change in Investigators	Added new Investigators. Attachments: relevant form FDA 1572s and CV.
6/13/2002	Protocol Amendment: New Investigator Protocol Amendment: Change in Investigators	Added new Investigators to protocol KL4-IRDS-02; Attachments: relevant form FDA 1572s and CVs.
6/14/2002	DSCO Telefax	Submitted IND Safety Report - 15 Day Alert (Initial).
6/14/2002	IND Safety Report: 15-Day Alert (Initial) Information Amendment: Clinical	Submitted IND Safety Report: 15-Day Alert (Initial).
7/1/2002	Protocol Amendment: New Investigators	Added new investigators to protocol KL4- IRDS-02 study; Attachments: relevant form FDA 1572s and CVs.
7/22/2002	DSCO Fax	Submitted IND Safety Report - 7 Day Alert (Initial).
7/26/2002	IND Safety Report - 7 Day Alert (Initial) Information Amendment: Clinical	Submitted IND Safety Report - 7 Day Alert (Initial). Amendment to the IB in the form of an Investigator Letter dated 22 July 2002.
8/19/2002	DSCO Fax	Submitted IND Safety Report - 7 Day Alert (Initial).
8/27/2002	DSCO Fax	Submitted IND Safety Report - 7 Day Alert (Initial).
9/5/2002	IND Safety Report - 7 Day Alert (Initial) Information Amendment: Clinical	Submitted IND Safety Report - 7 Day Alert (Initial). Amendment to the IB in the form of an Investigator Letter dated 15 August 2002.
9/5/2002	Protocol Amendment: New Investigators	Added new investigators to protocol KL4- IRDS-06. Attachments: relevant form FDA 1572s and CVs.
9/5/2002	Protocol Amendment: New Investigators	Added new investigators to protocol KL4-IRDS-02. Attachments: relevant form FDA 1572s and CVs.
9/23/2002	DSCO Fax	Submitted IND Safety Report - 7 Day Alert (Initial).
9/30/2002	Protocol Amendment: New Investigators	Added new investigators to protocol KL4-IRDS-06. Attachments: relevant form FDA 1572s and CVs.
10/14/2002	General Correspondence: Response to FDA Request for Information	Submitted CIOMS report for patient 793001 enrolled in protocol KL4-IRDS-06 as requested by FDA project manager, Yu, via telephone on September 26, 2002.

Date	*Type of Document*	*Description*
10/15/2002	General Correspondence	Requesting the Agency's approval to submit CIOMS reports for SAEs meeting expedited reporting requirement that occurred in protocols KL4-IRDS-06 and KL4-IRDS-02.
10/16/2002	IND Safety Report - 7 Day Alert (Initial) Information Amendment: Clinical	Submitted IND Safety Report - 7 Day Alert (Initial). Amendment to the IB in the form of an Investigator Letter dated October 8, 2002.
11/6/2002	Protocol Amendment: New Investigators Protocol Amendment: Change in Investigators	Added new investigator to protocol KL4-IRDS-02. Attachments: relevant form FDA 1572 and CV.
11/19/2002	Protocol Amendment: New Investigators Protocol Amendment: Change in Investigators	Added new investigators to protocol the KL4-IRDS-06. Attachments: relevant form FDA 1572s and CVs.
11/26/2002	DSCO Fax	Submitted IND Safety Report - 7 Day Alert (Initial).
11/27/2002	FDA TELEFAX	FDA teleconference meeting minutes from March 4, 2002 regarding status of protocol KL4-IRDS-06 and protocol changes submitted January 7, 2002.
12/20/2002	IND Safety Report: 7-Day Alert (Initial) Information Amendment: Clinical	Submitted IND Safety Report: 7-Day Alert (Initial). Amendment to the IB in the form of an Investigator Letter dated 19 December 2002.
1/9/2003	Protocol Amendment: New Investigator Protocol Amendment: Change in Investigator	Added new investigator to protocol KL4-IRDS-02. Attachments: relevant form FDA 1572 and CV.
1/13/2003	Annual Report	2002 Annual Safety Report- dated January 13, 2003
1/15/2003	DSCO Telefax	Reference is made to January 6, 8, and 10, 2003 telephone discussions regarding mortality in the KL4-IRDS-06 clinical trial.
1/20/2003	Protocol Amendment: New Investigator	Added new investigators to protocol KL4-IRDS-06. Attachments: relevant form FDA 1572s and CVs.
1/24/2003	Protocol Amendment: New Investigator	Added new investigator to protocol KL4-IRDS-02. Attachments: relevant form FDA1572 and CV.
2/20/2003	General Correspondence: Response to FDA Request for Info.	Reference is made to January 6, 8 and 10, 2003 discussions regarding mortality in KL4-IRDS-06. This information was previously sent to the FDA via fax on January 15, 2003. Provided data listing.
2/24/2003	FDA TELEFAX	Comments from April 29, 2002, serial no. 119 KL4-IRDS-06 submission. Clinical comments regarding the DAC SOP, statistical comments regarding the final SAP and the DSMB SOP.
2/26/2003	DSCO Telefax	Submitted IND Safety Report: 7-Day Alert (Initial).
2/27/2003	DSCO Telefax	Submitted IND Safety Report: 7-Day Alert (Initial).
2/28/2003	IND Safety Report: 7-Day Alert (Follow-up)	Submitted IND Safety Report: 7-Day Alert (Follow-up).
3/6/2003	IND Safety Report: 7-Day Alert (Follow-up)	Submitted IND Safety Report: 7-Day Alert (Initial).
3/12/2003	IND Safety Report: 7-Day Alert (Follow-up) Information Amendment: Clinical	Submitted IND Safety Report: 7-Day Alert (Follow-up). Amendment to the IB in the form of an Investigator Letter dated March 6, 2003.
3/12/2003	Information Amendment: Clinical	Amendment to the IB in the form of an Investigator Letter dated 6 March 2003. Reporting SAE.

Date	*Type of Document*	*Description*
3/12/2003	IND Safety Report: 7-Day Alert (Follow-up)	Submitted IND Safety Report: 7-Day Alert (Follow-up).
4/4/2003	FDA Letter	Letter from B. Chowdhury, Div. Director, requesting a plan of how Discovery will investigate the Agency's concern regarding the inactivation of Surfactant .
4/4/2003	FDA TELEFAX	Faxed Letter from B. Chowdhury, Div. Director, requesting a plan of how Discovery will investigate the Agency's concern regarding the inactivation of Surfactant.
4/8/2003	DSCO Telefax	To: Yu. From: Schaber. Regarding follow-up to phone conversation of April 7, 2003. Provided COA's for the following lots: 121231; 21492; 41332; and 51212. Additionally provided key points from previous discussions for the review team's consideration.
4/17/2003	Information Amendment: Clinical	Investigator brochure, version 8, dated April 15, 2003.
4/17/2003	General Correspondence: Response to FDA Request for Info.	March 28, 2003 correspondence with Mr. Arlyn Baumgarten, Director, FDA Chicago District Office regarding the status of Surfaxin® batches currently being used in trials; and April 8, 2003 fax that provided the agency with the COA's for the Surfaxin® batches under evaluation.
4/17/2003	General Correspondence: Request for Meeting	Request for Pre-NDA meeting for Surfaxin® in the prevention of RDS.
4/24/2003	DSCO Telefax	To: Yu. From: Rumage. Provided the April 29, 1998 letter from the United States Adopted Names Council (USAN) as follow-up to April 24, 2003 conversation.
4/24/2003	Information Amendment: Clinical	Final statistical analysis plan and table shells for protocol KL4-IRDS-02, entitled, "A Masked, Multicenter, Randomized, Controlled Trial Comparing the Safety and Effectiveness of Surfaxin® (lucinactant) to Curosurf® (poractant alfa) in the Prevention and Treatment of Respiratory Distress Syndrome (RDS) in Premature Neonates."
	General Correspondence: Response to FDA Request for Info.	The Agency's comments regarding protocol KL4-IRDS-06 - dated 02/24/2003 (fax copy); DSCO's response to the Agency's 02/24/2003 comments regarding protocol KL4-IRDS-06.
4/30/2003	General Correspondence	Briefing document for the Pre-NDA Meeting for Surfaxin® in RDS to be held on June 13, 2003 at the FDA Office.
5/16/2003	DSCO Telefax	Submitted IND Safety Report: 7-Day Alert (Initial).
5/30/2003	DSCO Telefax	Provided a response to the agency's letter dated April 4, 2003, received April 9, 2003 regarding the potential inactivation of surfactants when they come into contact wither rubber and/or rubber lubricant.
6/1/2003	DSCO Telefax	Submitted IND Safety Report: 7-Day Alert (Initial).
6/2/2003	DSCO Telefax	Follow-up to voice message on June 2, 2003 regarding the termination of enrollment of patients in protocol KL4-IRDS-02. Faxed consisted of a copy of the formal submission, serial no. 163.
6/2/2003	Information Amendment: Clinical	Notification to the FDA of termination of enrollment to protocol KL4-IRDS-02.
6/2/2003	General Correspondence: Response to FDA Request for Information	Agency's request dated 4/4/03; Discovery's response provided via fax 5/30/03, and the findings of E. Herting, G. Stichtenoth, and B. Robertson regarding rubber stoppers found in the Lancent, vol. 261, dated 1/25/03, pages 311-313.

Date	*Type of Document*	*Description*
6/3/2003	DSCO Telefax	Submitted IND Safety Report: 7-Day Alert (Initial) and IND Safety Report: 15-Day Alert (Initial).
6/4/2003	DSCO Telefax	Provided proposal for a change in manufacturing facility for Surfaxin® based on a recent FDA warning letter September 28, 2000 and FDA form 483s issued on December 17, 2001, August 30, 2002 and February 6, 2003.
6/4/2003	DSCO Telefax	Submitted IND Safety Report: 7-Day Alert (Initial).
6/6/2003	General Correspondence	Proposal for a change in manufacturing facility for Surfaxin® .
6/6/2003	IND Safety Report: 7-Day Alert (Follow-up) IND Safety Report: 15-Day Alert (Follow-up)	Submitted IND Safety Report: 7-Day Alert (Follow-up) and IND Safety Report: 15-Day Alert (Follow-up).
6/10/2003	Protocol Amendment: New Investigator Protocol Amendment: Change in Investigator	Added new investigators to protocol KL4-IRDS-06; Changes in sub-investigators at 11 sites. Attachments: relevant form FDA 1572s and CVs.
6/23/2003	DSCO Telefax	Submitted IND Safety Report: 7-Day Alert (Follow-up).
7/2/2003	IND Safety Report: 7-Day Alert (Follow-up)	Submitted IND Safety Report: 7-Day Alert (Follow-up).
7/8/2003	DSCO Telefax	Provided proposal for an animal model to address the agency's request for a toxicology study of Surfaxin®.
7/10/2003	Protocol Amendment: New Investigator Protocol Amendment: Change in Investigator	Added new investigators to Protocol KL4-IRDS-06; Attachments: relevant form FDA 1572s and CVs.
7/11/2003	DSCO Telefax	Submitted IND Safety Report: 7-Day Alert (Follow-up).
7/11/2003	FDA TELEFAX	FDA's meeting minutes for the June 13, 2003 pre-NDA meeting for Surfaxin in neonatal RDS.
7/11/2003	IND Safety Report: 7-Day Alert (Follow-up)	Submitted IND Safety Report: 7-Day Alert (Follow-up).
7/15/2003	Information Amendment: Clinical	Curriculum Vitae of a new Medical Monitor for Discovery Laboratories, Inc.
7/15/2003	General Correspondence Information Amendment: Pharmacology/Toxicology	Proposal to use a new animal model and supporting final nonclinical study reports.
7/17/2003	DSCO Telefax	Submitted IND Safety Report: 7-Day Alert (Follow-up).
7/23/2003	DSCO Telefax	Submitted IND Safety Report - 15 Day Alert (Follow-up).
7/23/2003	IND Safety Report: 7-Day Alert (Follow-up) IND Safety Report: 15-Day Alert (Follow-up)	Submitted IND Safety Report: 7-Day Alert (Follow-up) and IND Safety Report: 15-Day Alert (Follow-up).
7/24/2003	DSCO Telefax	Provided proposal to submit the NDA.
7/31/2003	DSCO Telefax	Submitted IND Safety Report - 15 Day Alert (Follow-up # 2).
7/31/2003	DSCO Telefax	Provided proposal to an animal model for the agency requested toxicology study for Surfaxin®.
7/31/2003	General Correspondence	Proposal to submit the Surfaxin® new drug application (NDA).
8/8/2003	DSCO Telefax	Submitted IND Safety Report: 7-Day Alert (Follow-up)..
8/11/2003	DSCO Telefax	Provided Discovery's proposal for the analysis of the co-primary endpoints for protocol KL4-IRDS-06.
8/12/2003	General Correspondence	Proposal to use an animal model for the animal toxicology study of Surfaxin®.

Date	*Type of Document*	*Description*
8/15/2003	Information Amendment: Clinical	Amendment to the IB in the form of an Investigator Letter dated 4 August 2003.
8/15/2003	IND Safety Report: 7-Day Alert (Follow-up) IND Safety Report: 15-Day Alert (Follow-up)	Submitted IND Safety Report: 7-Day Alert (Follow-up) and IND Safety Report: 15-Day Alert (Follow-up).
8/19/2003	General Correspondence	Proposal for analysis of the co-primary endpoints for KL4-IRDS-06; A copy of the agency's meeting minutes from the pre-NDA meeting held on June 13, 2003; and Discovery's meeting minutes from the pre-NDA meeting held on June 13, 2003.
8/22/2003	DSCO Telefax	Submitted IND Safety Report: 7-Day Alert (Initial).
8/27/2003	DSCO Telefax	Submitted IND Safety Report: 7-Day Alert (Follow-up) and IND Safety Report: 15-day (Initial).
8/27/2003	IND Safety Report: 7-Day Alert (Initial)	Submitted IND Safety Report: 7-Day Alert (Initial).
8/28/2003	IND Safety Report: 7-Day Alert (Follow-up)	Submitted IND Safety Report: 7-Day Alert (Follow-up).
9/4/2003	IND Safety Report: 15-Day Alert (Initial)	Submitted IND Safety Report: 15-Day Alert (Initial).
9/8/2003	DSCO Telefax	Submitted IND Safety Report: 7-day Alert (Follow-up) and IND Safety Report: 15-day Alert (Follow Up).
9/12/2003	Protocol Amendment: New Investigator Protocol Amendment: Change in Investigator	Added new investigators to protocol KL4-IRDS-06. Attachments: relevant form FDA 1572s and CVs.
9/19/2003	IND Safety Report: 7-Day Alert (Follow-up) IND Safety Report: 15-Day Alert (Follow-up) Information Amendment: Clinical	Submitted IND Safety Report: 7-Day Alert (Follow-up) and IND Safety Report: 15-Day Alert (Follow-up). Amendment to the IB in the form of an Investigator Letter dated 11September2003.
9/25/2003	DSCO Telefax	Submitted IND Safety Report: 7-Day Alert (Follow-up).
9/25/2003	DSCO Telefax	To: Yu. From: Rumage. Provided a new proposal to use a new animal model.
9/25/2003	General Correspondence	Proposal to use a new animal model for the animal toxicology study of Surfaxin®.
10/1/2003	DSCO Telefax	Submitted IND Safety Report: 7-Day Alert (Follow-up) and IND Safety Report: 15-Day Alert (Follow-up).
10/3/2003	DSCO Telefax	Submitted IND Safety Report: 7-Day Alert (Initial).
10/3/2003	DSCO Telefax	Provided Discovery's proposal for the Surfaxin® drug product stability data to be included in the new drug application (NDA).
10/3/2003	General Correspondence	Proposal for the Provision of Drug Product Stability for the New Drug Application (NDA) Filing for Surfaxin® in Respiratory Distress Syndrome (RDS).
10/13/2003	DSCO Telefax	Submitted IND Safety Report: 15-day Alert (Follow-up).
10/14/2003	FDA TELEFAX	Faxed comments from the FDA (Christine Yu) to Discovery (Christopher Schaber) in response to our proposals for IND 40,287 (serial nos. 174, 175, and 184) regarding submitting the NDA.
10/17/2003	Annual Report	2003 Annual Safety Report- dated October 17, 2003
10/21/2003	DSCO Telefax	Submitted IND Safety Report: 7-Day Alert (Follow-up).
10/21/2003	IND Safety Report: 7-Day Alert (Follow-up)	Submitted IND Safety Report: 7-Day Alert (Follow-up).

Date	*Type of Document*	*Description*
10/21/2003	IND Safety Report: 7-Day Alert (Follow-up) IND Safety Report: 15-Day Alert (Follow-up) Information Amendment: Clinical	Submitted IND Safety Report: 7-Day Alert (Follow-up) and IND Safety Report: 15-Day Alert (Follow-up).
10/21/2003	IND Safety Report: 7-Day Alert (Initial) Information Amendment: Clinical	Submitted IND Safety Report: 7-Day Alert (Initial). Amendment to the IB in the form of an Investigator Letter dated 7 October 2003.
10/30/2003	General Correspondence	Supplemental information for the proposal for the analysis of the co-primary endpoints for protocol KL4-IRDS-06.
10/31/2003	DSCO Telefax	Discovery (C. Schaber) fax to the FDA (C. Yu) - provided supplement to August 19, 2003 Co-primary endpoint proposal along with October 29, 2003 from the Data Monitoring Committee.
11/6/2003	DSCO Telefax	Submitted IND Safety Report: 7-Day Alert (Follow-up) and IND Safety Report: 15-day Alert (Follow up).
11/7/2003	FDA TELEFAX	FDA comments in response to serial no. 185 for IND 40,287.
11/12/2003	Protocol Amendment: Change in Investigator	Change in principal investigator for protocol KL4-IRDS-06;Attachments: relevant form FDA 1572 and CV.
11/12/2003	IND Safety Report: 7-Day Alert (Follow-up) IND Safety Report: 15-Day Alert (Follow-up)	Submitted IND Safety Report: 7-Day Alert (Follow-up) and IND Safety Report: 15-Day Alert (Follow-up).
11/14/2003	FDA TELEFAX	FDA comments in response to serial nos. 178 and 190 for IND 40,287.
11/19/2003	Information Amendment: Clinical	Updated Surfaxin® Investigator Brochure, Version 9, dated November 7, 2003 for the IRDS and MAS indications.
11/20/2003	Protocol Amendment: Change in Protocol Information Amendment: Clinical	FDA's Nov. 14, 2003 facsimile transmission; Change document for Protocol KL4-IRDS-06 Amendment 3; Protocol KL4-IRDS-06 Amendment 3-dated November 19, 2003; DSMB SOP Amendment 1 – dated Nov. 17, 2003; Original DSMB SOP - dated Jan. 31, 2002.
11/21/2003	DSCO Telefax	Submitted IND Safety Report: 7-Day Alert (Follow up).
11/21/2003	Information Amendment: Clinical	Amended Statistical Analysis Plan for protocol KL4-IRDS-06 revised to address changes in the primary and secondary endpoints per the agency's comments from the June 13, 2003 pre-NDA meeting.
11/24/2003	IND Safety Report: 7-Day Alert (Follow-up)	Submitted IND Safety Report: 7-Day Alert (Follow-up).
11/25/2003	Information Amendment: Clinical	Official notification to the FDA to stop patient enrollment into the KL4-IRDS-06 clinical trial based on the discretion of the DSMB due that the target number of events had been reached for the co-primary endpoints for the study.
11/29/2003	Information Amendment: Clinical	Cover letter dated November 22, 2003 from the DSMB (Joe Massaro. PhD) to Badrul Chowdhury, MD, PhD in which the DSMB provided the revised SAP for protocol KL4-IRDS-06.
12/4/2003	Information Amendment: Clinical	Revised Adjudication SOP manual for Protocol KL4-IRDS-06 Amendment 2- dated November 19, 2003.
1/6/2004	DSCO Telefax	Submitted IND Safety Report: 7 Day Alert (Follow up) and IND Safety Report: 15- DAY (Follow up).

Date	*Type of Document*	*Description*
1/7/2004	IND Safety Report: 7-Day Alert (Follow-up) IND Safety Report: 15-Day Alert (Follow-up)	Submitted IND Safety Report: 7-Day Alert (Follow-up) and IND Safety Report: 15-Day Alert (Follow-up).
1/12/2004	DSCO Telefax	To: Yu. From: Rumage. Proposal for RDS NDA.
1/16/2004	IND Safety Report: 7-Day Alert (Follow-up)	Submitted IND Safety Report: 7-Day Alert (Follow-up).
1/20/2004	DSCO Telefax	Submitted IND Safety Report: 7-Day Alert (Follow-up).
1/31/2004	DSCO Telefax	Submitted IND Safety Report: 7-Day Alert (Follow-up).
2/3/2004	DSCO Telefax	Submitted IND Safety Report: 7-Day Alert (Follow-up).
2/3/2004	IND Safety Report: 7-Day Alert (Follow-up)	Submitted IND Safety Report: 7-Day Alert (Follow-up).
2/11/2004	DSCO Telefax	Submitted IND Safety Report: 7-Day Alert (Follow-up).
2/11/2004	Information Amendment: Clinical	Change document for KL4-IRDS-06 SAP , version 5; KL4-IRDS-06 Statistical Analysis Plan , version 5 (2 volumes).
3/8/2004	DSCO Telefax	Submitted IND Safety Report: 7-Day Alert (Follow-up).
3/8/2004	IND Safety Report: 7-Day Alert (Follow-up)	Submitted IND Safety Report: 7-Day Alert (Follow-up).
3/11/2004	IND Safety Report: 7-Day Alert (Follow-up)	Submitted IND Safety Report: 7-Day Alert (Follow-up).
3/16/2004	Protocol Amendment: Change in Investigator	Changes in principal investigators and sub-investigators for protocol KL4-IRDS-02. Attachments: relevant form FDA 1572s and CVs.
3/22/2004	Protocol Amendment: Change in Investigator	Changes in sub-investigators at 11 sites for Protocol KL4-IRDS-06.
3/23/2004	DSCO Telefax	Submitted IND Safety Report.
3/23/2004	DSCO Telefax	To: Yu. From: Rumage. Questions to the FDA regarding the submission of IRDS NDA # 21,746.
4/5/2004	DSCO Telefax	Submitted IND Safety Report 7-DAY Alert (Follow-up).
4/19/2004	Information Amendment: Chemistry/Microbiology	CMC amendment to allow for a change in manufacturing facility.
4/23/2004	FDA Telefax	To: World Courier, Inc. From: R. Meja (FDA) Provided notice of FDA Action, entry no. 113-2657439-6 regarding the importation of study drugs.
4/30/2004	IND Safety Report: 7-Day Alert (Follow-up)	IND Safety Report: 7-Day Alert (Follow-up).
5/18/2004	DSCO Telefax	To: R. Meja From: K. Tsokas Response to April 23, 2004 notice of FDA Action, entry no. 113-2657439-6 for clinical study drugs.
5/24/2004	FDA Telefax	To: World Courier, Inc. From: R. Meja (FDA) Provided notice of FDA Action to release Exosurf®.
6/15/2004	FDA Telefax	Submitted IND Safety Report: 7-Day Alert (Follow up).
6/16/2004	IND Safety Report: 7-Day Alert (Follow-up)	Submitted IND Safety Report: 7-Day Alert (Follow-up).
6/25/2004	DSCO Telefax	Submitted IND Safety Report: 7-Day Alert (Follow-up).
6/29/2004	IND Safety Report: 7-Day Alert (Follow-up)	Submitted IND Safety Report: 7-Day Alert (Follow-up).
7/2/2004	DSCO Telefax	Submitted IND Safety Report: 7-Day Alert (Follow-up).
7/6/2004	IND Safety Report: 7-Day Alert (Follow-up)	Submitted IND Safety Report: 7-Day Alert (Follow-up).

Date	*Type of Document*	*Description*
7/28/2004	DSCO Telefax	Submitted IND Safety Report: 7- Day Alert (Follow-up).
7/29/2004	Information Amendment: Clinical	Text portion of the KL4-IRD-02 clinical study report dated March 15, 2004 along with appendix 16.1.9 (Statistical Analysis Plan). (2 volumes).
7/30/2004	Information Amendment: Clinical	Text portion of the KL4-IRD-06 clinical study report dated March 21, 2004 along with appendix 16.1.9 (Statistical Analysis Plan). (2 volumes).
8/4/2004	IND Safety Report: 7-Day Alert (Follow-up)	Submitted IND Safety Report: 7-Day Alert (Follow-up).
8/26/2004	General Correspondence	Proposal for a 3b clinical protocol for the treatment of premature infants with RDS; Request for Teleconference if necessary.
10/6/2004	Information Amendment: Clinical	Curriculum Vitae of new Medical Monitor for IND 40,287.
10/22/2004	Protocol Amendment: New Protocol	New Protocol, KL4-BPD-01 entitled "A Randomized, Double-blind, Placebo-Controlled Trial to Assess the Safety and Efficacy of Surfaxin® (lucinactant), in Very Low Birth Weight (VLBW) Infants at Risk for Developing Bronchopulmonary Dysplasia".
11/17/2004	DSCO Telefax	Submitted IND Safety Report: 7-Day Alert (Follow up) and IND Safety Report: 15-Day Alert (Follow up).
11/17/2004	General Correspondence	Notification of change of address, telephone number, and fax number for Discovery's corporate office.
11/18/2004	Annual Report	2004 Annual Safety Report covering the period August 1, 2003 through July 31, 2004 (2 volumes).
11/24/2004	FDA Telefax FDA Minutes	Agency's meeting minutes from October 27, 2004 teleconference regarding clarification on issues arising from two submissions to IND 40,287 and NDA 21-746.
11/30/2004	IND Safety Report: 7-Day Alert (Follow-up) IND Safety Report: 15-Day Alert (Follow-up)	Submitted IND Safety Report: 7-Day Alert (Follow-up) and IND Safety Report: 15-Day Alert (Follow-up).
12/29/2004	Information Amendment: Pharmacology/Toxicology	Notification of the transfer of documents/specimens.
1/11/2005	Information Amendment: Pharmacology/Toxicology	Amendment final study reports.
1/14/2005	Information Amendment: Clinical	Submitted CIOMS reports for the following patients enrolled in KL4-IRDS-02: 201002, 211001, 211002, 212002, 221002, 302006, 702005, 711002, 731003, and 812003.
2/2/2005	DSCO Telefax	Submitted IND Safety Report 15-Day Alert: (Follow up).
2/2/2005	IND Safety Report: 15-Day Alert (Follow-up)	Submitted IND Safety Report: 15-Day Alert (Follow-up).
3/11/2005	Information Amendment: Clinical	Change in medical monitor.
4/19/2005	Information Amendment: Clinical	Provided CIOMS reports for the following patients enrolled in KL4-IRDS-06 who experienced SAEs that met expedited reporting criteria: 012003, 043007, 062002, 063001, 063005, 082001, 201002, 553003, 701004, 731001, 732002, 751012, and 782002.
5/4/2005	Protocol Amendment: Change in Investigators Protocol Amendment: Deleted Investigators Information Amendment: Clinical	Final change in investigators for protocols KL4-IRDS-02 and KL4-IRDS-06.

Date	*Type of Document*	*Description*
8/26/2005	Information Amendment: Clinical	Text portion of the Amended short term and long term Clinical Study Reports for KL4-IRDS-02 and KL4-IRDS-06; KL4-IRDS-02 Volume 1 of 2; KL4-IRDS-06 Volume 2 of 2.
1/24/2006	Annual Report	2005 Annual Report covering the period August 1, 2004 through July 31, 2005.
12/18/2006	Annual Report	2006 Annual Report covering the period August 1, 2005 through September 4, 2006.
3/20/2007	Information Amendment: Chemistry, Manufacturing, and Controls	CMC Amendment in CTD format to support clinical trials using 30 mg/mL drug product. (3 Volumes)
5/11/2007	Information Amendment: Pharmacology/Toxicology	Meeting Minutes from the 21DEC2006 Type C meeting for NDA 21-746; Discovery's proposal for the conduct of a SURFAXIN impurity qualification study in ferrets; Literature references
6/26/2007	FDA Telefax	FDA comments regarding pharmacology/toxicology proposal submitted May 11, 2007 (Serial No. 295).
1/4/2008	Annual Report	2007 Annual Report covering September 5, 2006 - September 4, 2007. IB Edition 11 (December 11, 2007) included as an attachment.
1/4/2008	Information Amendment: Pharmacology/Toxicology	Two Nonclinical Study Reports: Bolus Delivery of Lucinactant in Ventilated Preterm Lambs and Acute Intra-Tracheal Instillation Toxicity Study of Lucinactant Impurities.
10/30/2008	Annual Report	2008 Annual Report covering the period September 5, 2007 through September 4, 2008
11/21/2008	Information Amendment: Pharmacology/Toxicology	Preclinical study entitled "Effect of Intratracheal Lucinactant on Respiratory System Compliance in Ventilated Very Preterm Lambs"

SURFAXIN® NDA 21-746 Brief Description of Significant Events

Date	*Type of Submission*	*Description of Submission*
4/13/2004	Original NDA Application	Discovery Labs filed the original NDA application for SURFAXIN.
4/14/2004	Original NDA Application - Field Copy	Provided the field copy to the North Brunswick Field Office.
4/26/2004	DSCO Facsimile Response to FDA Request for Additional Information	Provided via facsimile the October 18, 1995 letter granting the Scripps Research Institute Orphan Drug Designation for KL_4 Surfactant (currently known as SURFAXIN) to be included in Module 1, Section 1.5 of NDA 21-746 as follow-up to the April 26, 2004 phone conversation with C. Yu (FDA).
4/27/2004	General Correspondence Response to FDA Request for Additional Information	Provided the October 18, 1995 letter granting the Scripps Research Institute Orphan Drug Designation for KL_4 Surfactant (currently known as SURFAXIN) to be included in Module 1, Section 1.5 of NDA 21-746 as follow-up to the April 26, 2004 facsimile.
4/28/2004	General Correspondence Response to FDA Request for Additional Information	Provided the North Brunswick Field Office with the October 18, 1995 letter granting the Scripps Research Institute Orphan Drug Designation for KL_4 Surfactant (currently known as SURFAXIN) to be included in Module 1, Section 1.5 of NDA 21-746.
5/17/2004	General Correspondence Response to FDA Request for Additional Information	Provided response to the May 14, 2004 request for information to the Division of Scientific Investigations. Documents for KL4-IRDS-06 submitted were: Protocol & amends; Blank case report form; Sample ICF; Description of primary endpoints; Table 1.A; Table 1.B; Table 2.A; Table 2.B. (2 volumes).

Date	*Type of Submission*	*Description of Submission*
5/17/2004	FDA Correspondence	Original receipt for the NDA application for NDA 21-746 (received date 05/21/2004). Also, facsimile receipt for the NDA application for NDA 21-746 (received date 05/18/2004).
5/24/2004	General Correspondence Response to FDA Request for Additional Information DSCO Facsimile	Provided response to the May 21, 2004 request for information to the Division of Scientific Investigations.
6/11/2004	DSCO Facsimile	Provided contact Information for the principal investigators at sites 8, 30, 72 and 75 as requested by the FDA.
6/15/2004	General Correspondence Response to FDA Request for Additional Information	Provided response to June 4, 2004 request for five desk copies of Module 1, and the original NDA cover letter and Form FDA 356h on CD-ROM in PDF.
6/15/2004	General Correspondence Response to FDA Request for Additional Information	Provided requested information from June 9, 2004 to the Division of Scientific Investigations.
6/16/2004	DSCO Facsimile	Copy of the cover letter regarding: Requested information from June 9, 2004 to the Division of Scientific Investigations was forwarded via facsimile to C. Yu (FDA)
6/22/2004	DSCO Correspondence Response to FDA Request for Additional Information	Provided the Philadelphia District Office with a CD-ROM of the CMC portion (Module 3) of NDA 21-746-dated 4/13/2004.
6/25/2004	FDA Correspondence	Original 74-day letter from the Division. Acceptance of NDA 21-746 (June 12, 2004) received date 07/01/2004. Faxed copy of Original 74-day letter from the Division(received date 06/28/2004).
6/29/2004	DSCO Correspondence	Provided requested information from June 23, 2004 regarding CMC information.
7/1/2004	DSCO Correspondence	Provided request for the division's acceptance of proposed submission of clinical information.
7/15/2004	FDA Facsimile FDA Request for Additional Information	Request for clinical information for patients enrolled in protocol KL4-IRDS-06 in a tabular format preferably for all three study drugs, Exosurf®, Survanta®, and SURFAXIN®.
7/20/2004	DSCO Facsimile FDA Request for Additional Information	Provided a template of the listings of SAEs through 36 weeks PCA, day of withdrawal of consent, deaths, date of onset, SOC/PT, and listing of primary endpoint related data from CRF and Adjudication results for sites 08, 30, 72, & 75.
7/29/2004	General Correspondence Response to FDA Request for Additional Information	Provided two copies of the following information: primary endpoint related data; treatment assignment; and SAEs through 36 weeks PCA, day of withdrawal of consent, or death for sites 08, 30, 72, and 75.
8/10/2004	DSCO Correspondence FDA Request for Additional Information	Provided R. Rodriguez (FDA -Puerto Rico) with a copy of Module 3 on CD-ROM. Additionally, provided a copy of the cover letter that was sent to R. Rodriguez to C. Yu (FDA) and L. Adams (DFI) via facsimile.
8/16/2004	Form FDA 483	Form FDA 483 for the inspection that occurred from August 10, 2004 - August 16, 2004.
8/16/2004	General Correspondence Response to FDA Request for Additional Information	Provided a response to the agency's July 15, 2004 request for additional information which includes a tabular summary of requested endpoints by batch for protocol KL4-IRDS-06.
8/18/2004	FDA Letter	Letter from the Philadelphia District office regarding

Date	*Type of Submission*	*Description of Submission*
		Discovery's Doylestown's facility.
8/24/2004	DSCO Facsimile Response to FDA Request for Additional Information	Provided contact information for inspection at the Doylestown office via facsimile.
9/3/2004	General Correspondence Response to FDA Request for Additional Information	Provided a response to DSI's August 25, 2004 request for additional clinical information: patient ID, birth date and time, gestational age, birth weight, and date and time of dose for sites 08, 30, 72, and 75.
9/13/2004	General Correspondence Response to Form FDA 483	Provided a response to the Form FDA 483 issued on August 16, 2004 to both the Philadelphia District Office (T. Gardine) and the Maryland Office (B. Chowdhury).
9/14/2004	FDA Letter	Original receipt from the Philadelphia District Office for Discovery's September 13, 2004 response to the Form FDA 483 issued on August 16, 2004.
9/24/2004	E-Mail Correspondence	The FDA requested additional information regarding the DSMB's final SOP and requested the interim statistical analyses reports for the co-primary endpoints for the NDA.
9/28/2004	DSCO Facsimile Response to FDA Request for Additional Information	Provided with the original KL4-IRDS-06 Adjudication Committee Manual SOP and four representative ballots utilized by the Adjudication Committee via facsimile.
9/30/2004	DSCO Facsimile Response to FDA Request for Additional Information	Provided a sample adjudication data tracking form and a sample data listing that identifies with Adjudication Committee members voted on each patient.
9/30/2004	General Correspondence	Provided Safety Update and Clinical Update.
10/4/2004	DSCO Facsimile Response to FDA Request for Additional Information	Provided FDA LA District Office with a copy of analytical test method, DP-018.
10/4/2004	DSCO Facsimile Response to FDA Request for Additional Information	Provided a data listing which provides the details the patients evaluated by each adjudicator as part of the process of adjudicating the endpoints for the KL4-IRDS-06 study.
10/7/2004	DSCO Facsimile Response to FDA Request for Additional Information	Provided an updated list of patients evaluated by each adjudicator and a change document for the Adjudication Committee manual SOP.
10/8/2004	FDA Letter Response to FDA Request for Additional Information	Provided a response to the September 24, 2004 e-mail regarding the DSMB SOP and reports detailing the conduct and results of any interim analyses of the co-primary endpoints of the KL4-IRDS-06 study.
10/19/2004	General Correspondence/CMC Amendment	Provided DSCO's response to the Division's 74 Letter dated June 25, 2004.
10/21/2004	FDA Letter	Form FDA 483 issued for inspection held on October 13, 15, 18, and 21, 2004.
10/22/2004	DSCO Facsimile Response to FDA Request for Additional Information	Provided a list of hotel accommodations and transportation services for the upcoming inspections. (Note: Date on fax transmittal form is incorrectly recorded as 10/22/2003 s/b 10/22/2004)
10/28/2004	DSCO Facsimile Response to FDA Request for Additional Information	Provided certifications for the four sites who are going to be audited (08, 30, 72, and 75) stating they are available on the scheduled dates of inspection.
11/1/2004	General Correspondence Response to FDA Request for Additional Information DSCO Facsimile	Provided relevant information for the DSMB in response to the agency request from the October 27, 2004 teleconference. (3 Volumes)
11/3/2004	General Correspondence Response to FDA Request for	Provided two replacement pages to the November 1, 2004 DSMB submission. A copy of the cover letter was also

Date	*Type of Submission*	*Description of Submission*
	Additional Information DSCO Facsimile	provided via facsimile to C. Yu (FDA).
11/15/2004	General Correspondence Response to Form FDA 483	Provided additional information for the response to the Form FDA 483 issued on August 16, 2004 to both the Philadelphia District Office and the Division. Initial response was provided September 13, 2004.
11/17/2004	General Correspondence	Notification of Change of Corporate Address to 2600 Kelly Road, Warrington, PA 18976
11/22/2004	FDA Facsimile FDA Request for Additional Information	Request for additional information for the patients for the KL4-IRDS-06 study.
11/23/2004	General Correspondence Response to Form FDA 483	Provided a response to the Form FDA 483 issued on November 2, 2004. Copy provided to the Division and the LA District Office.
11/24/2004	FDA Meeting Minutes	Agency's meeting minutes from October 27, 2004 teleconference.
11/24/2004	DSCO Facsimile Response to FDA 483	Provided November 23, 2004 cover letter in response the FDA 483 issued on November 2, 2004.
11/24/2004	General Correspondence FDA Facsimile Response to FDA 483	Provided a response regarding additional information requested during the inspection held at Discovery from Nov. 8-10, 2004.
11/29/2004	General Correspondence Response to FDA Request for Additional Information	Provided a complete copy of the submission of November 24, 2004 which included a description of actions taken at three adjudication meetings, a list of patients adjudicated at the corresponding meetings, and copies of 17 requested patient files. (2 Volumes).
12/1/2004	Response to FDA Request for Additional Information	Provided a response the FDA's November 22, 2004 facsimile regarding additional information for patients who died by day 14 regarding relationship to RDS.
12/1/2004	Response to FDA Request for Additional Information Response to Form FDA 483	Provided a complete copy of the November 15, 2004 submission.
12/1/2004	DSCO Facsimile	Provided a copy of the November 24, 2004 letter to DSI in response to additional items requested during the agency's visit November 8-10, 2004.
12/8/2004	General Correspondence Clarification to NDA Submission Dated April 13, 2004	Provided a list the SAS decodes/codes of countries in KL4-IRDS-02 and KL4-IRDS-06.
12/8/2004	General Correspondence Clarification to NDA Submission Dated December 1, 2004	Provided the electronic document room with a copy of the SAS Data Transport File provided on December 1, 2004. CD-ROM also included cover letters, 356h form, and corresponding SAS decodes/codes.
12/8/2004	General Correspondence Clarification to NDA Submission Dated December 1, 2004	Provided a list the SAS decodes/codes of the adjudicators and relationship of death to RDS.
12/9/2004	DSCO Facsimile	Provided a copy of the 3 cover letters submitted on December 8, 2004 in response to the December 7, 2004 telephone request.
12/14/2004	FDA Facsimile FDA Request for Additional Information	Request for additional information regarding the process of the adjudication committee's votes.
12/17/2004	General Correspondence	Provided clarification for the occurrence of a committee vote

Date	*Type of Submission*	*Description of Submission*
	DSCO Facsimile Clarification to NDA Submission Dated December 1, 2004	when 2 adjudicators agreed and clarification for two contradictory votes from the same adjudicator. Copy of cover letters also sent via facsimile to C. Yu (FDA).
12/20/2004	General Correspondence Response to FDA Request for Additional Information	Provided Dr. Chowdhury a letter stating that new CD-ROMS were provided to the EDR.
12/20/2004	General Correspondence Response to FDA Request for Additional Information	Provided new CD-ROMS to the EDR as clarification to 11/29/04 submission
12/21/2004	DSCO Facsimile	Provided copies of submissions sent on 12/20/04 to the EDR and Dr. Chowdhury.
12/21/2004	Form FDA 483	Form FDA 483 for Dec. 20 - Dec. 21, 2004 inspection at Doylestown facility.
12/28/2004	General Correspondence Response to FDA Request for Additional Information	Provided the Electronic Document Room (EDR) with a copy of the Draft Package Insert in Word. Additionally, provided the Division with a copy of the cover letter sent to the EDR.
12/29/2004	General Correspondence	Provided notification to the agency of the transfer of specimens and documentation from Provident Preclinical, Inc. to Discovery and EPL Archives.
12/29/2004	DSCO Facsimile Response to FDA Request for Additional Information	Provided copies of the cover letters submitted to the EDR and Pulmonary Division on December 28, 2004.
1/4/2005	General Correspondence Clarification to Original NDA Submission Dated April 13, 2004 DSCO Facsimile	Provided notification to the agency that DMF No. 17159 was updated as requested on Dec. 9, 2004. Also provided a copy of the cover letter via fax.
1/6/2005	DSCO Facsimile	Provided a copy of the cover letter for Discovery's January 4, 2005 submission to NDA 21-746 regarding the amended DMF No. 17159
1/6/2005	FDA Facsimile	Request for clarification regarding the differences of abnormal findings in protocols KL4-IRDS-02 and KL4-IRDS-06; additionally, whether or not ultrasounds were used.
1/6/2005	General Correspondence Response to Form FDA 483	Provided a response to the Form FDA 483 issued on Dec. 21, 2004 to both the Philadelphia District Office and the Pulmonary Division.
1/7/2005	DSCO Facsimile	Provided copies of the cover letters provided to both the Philadelphia District Office and the Pulmonary Division in response to the Form FDA 483 issued on Dec. 21, 2004.
1/7/2005	FDA Letter Establishment Inspection Report	Provided a copy of the establishment inspection report for the inspection conducted at UCSD from November 1 - November 2, 2004.
1/10/2005	General Correspondence Response to FDA Request for Additional Information DSCO Facsimile	Discovery's response to the agency's Jan. 6, 2005 facsimile and Jan. 10, 2005 telephone conversation regarding the differences of abnormal findings in protocols KL4-IRDS-02 and KL4-IRDS-06; additionally, whether or not ultrasounds were used. Also provided a copy of the submission via facsimile. (copy of the agency's Jan. 6, 2005 facsimile included).
1/12/2005	General Correspondence Response to Form FDA 483	Provided a response to the Form FDA 483 issued on Dec. 9, 2004 to both the Philadelphia District Office and the Pulmonary Division.
1/12/2005	DSCO Facsimile	Resent Discovery Jan. 10, 2005 fax which included Discovery's response to the comments received from the

Date	*Type of Submission*	*Description of Submission*
		agency on Jan. 6, 2005 regarding the differences of abnormal findings in protocols KL4-IRDS-02 and KL4-IRDS-06; additionally, whether or not ultrasounds were used.
1/13/2005	FDA Letter General Correspondence	Acknowledgement of receipt of Discovery's January 12, 2005 submission to the Philadelphia District Office regarding DSCO responses to the FDA Form 483
1/14/2005	FDA Facsimile FDA Meeting Minutes	FDA Meeting Minutes from January 10, 2005 Teleconference.
1/31/2005	General Correspondence Response to FDA 483 Laureate Letter	Provided a response to the Form 483 issued on January 21, 2005 to the Parsippany, NJ Field Office.
1/31/2005	General Correspondence Response to Form FDA 483 DSCO Facsimile	Provided a copy of response to the Form 483 issued on January 21, 2005 to the Pulmonary Division. Additionally, provided a copy of cover letters along with attachments 1 and 2 via facsimile.
2/3/2005	FDA Letter Establishment Inspection Report	Provided a copy of the establishment inspection report for the inspections conducted August 10 - 16, and December 20 - 21, 2004 at Discovery's Doylestown facility.
2/11/2005	General Correspondence	Response to FDA meeting minutes from January 10, 2005 teleconference.
2/11/2005	FDA Facsimile	Provided approvable letter for NDA 21-746 which includes deficiencies noted during the review process.
2/11/2005	FDA Letter	Provided approvable letter for NDA 21-746 which includes deficiencies noted during the review process.
2/17/2005	General Correspondence	Notification of intent to file an amendment to NDA 21-746 in response to the February 11, 2005 approvable letter.
2/17/2005	DSCO Facsimile	Notification of intent to file an amendment to NDA 21-746 in response to the February 11, 2005 approvable letter.
3/18/2005	General Correspondence FDA Facsimile	Request for clarification to the approvable letter dated February 11, 2005 from the agency. Cover letter also sent via facsimile.
3/20/2005	General Correspondence FDA Letter	Comments from the FDA regarding the SOP Manual for the KL4-IRDS-06 Adjudication Committee (Nov. 19, 2003) and request for corrections.
3/31/2005	General Correspondence	Update to manufacturing activities.
4/1/2005	General Correspondence	Provided Discovery's clarification and proposal to the Agency's February 11, 2005 approvable letter regarding the CMC section of NDA 21-746.
4/2/2005	DSCO Facsimile	Provided a copy of the cover letter and Discovery's clarification and proposal to the Agency's February 11, 2005 approvable letter regarding the CMC section of NDA 21-746.
4/8/2005	General Correspondence DSCO Facsimile DSCO E-mail	Request for the division's acceptance of proposed submission of clinical information for the safety update. Cover letter sent via e-mail and facsimile.
5/11/2005	FDA Letter	FDA's response to submission dated 04/08/2005- submission of clinical information for safety update
6/8/2005	General Correspondence DSCO Facsimile	Request for a teleconference to discuss April 1, 2005 CMC points of clarification document sent via courier and fax.
6/17/2005	General Correspondence FDA Letter	Notification of date and time of CMC points of clarification teleconference: July 29, 2005 (11:00 AM - 12:00 PM), a tentative list of attendees. 12 copies of meeting package requested.

Date	*Type of Submission*	*Description of Submission*
7/6/2005	General Correspondence	Provided copies of the July 29, 2005 Meeting Package regarding the CMC section of NDA 21-746.
7/13/2005	General Correspondence Response to FDA Request for Additional Information	Provided a copy of response to the remaining items noted on the Form 483 dated January 21, 2005.
7/27/2005	General Correspondence DSCO Facsimile	Discovery's request to cancel the July 29, 2005 teleconference to discuss the CMC section of NDA 21-746 sent via FedEx and fax.
7/29/2005	PDUFA Response	Provided the North Brunswick Field Office with a copy of Volumes 1, 153-157.
7/29/2005	PDUFA Response	Provided a response to the February 11, 2005 PDUFA letter (157 Volumes) to the Pulmonary Division. Copy of cover letters sent to C. Yu via facsimile.
8/16/2005	FDA Facsimile	FDA Comments regarding Discovery's July 29, 2005 PDUFA response.
10/5/2005	General Correspondence DSCO Facsimile	Provided the complete PDUFA response in response the approvable letter dated February 11, 2005 and the August 16, 2005 facsimile to the Agency and the field office.
10/20/2005	FDA Facsimile FDA Letter	Acknowledgment of the October 5, 2005 PDUFA response, which is considered a class 2 response to the February 11, 2005 approvable letter. The user fee goal date is April 6, 2006.
10/24/2005	General Correspondence	DSCO response to the FDA request for additional copies of 10/05/05 Resubmission- Complete Response to NDA Approval Letter-dated 02/11/05 and 08/16/05 FDA Fax.
11/2/2005	General Correspondence	DSCO response to the FDA request for additional information.
11/22/2005	FDA Facsimile	Request for annotated amended clinical study reports for KL4-IRDS-02 and KL4-IRDS-06 as well as the ISE, or written guides specifying the changes in the clinical study reports and ISE submitted in the PDUFA response.
11/23/2005	General Correspondence DSCO Facsimile	Provided a type C meeting request to gain agreement with the Agency regarding the appropriateness of data submitted in Discovery's October 5, 2005 PDUFA response in relation to drug product impurity qualification and analytical methodology and confirm there is no additional issues pending with the NDA.
12/2/2005	FDA Facsimile FDA Letter	Notification that our November 23, 2005 meeting request would not be considered productive at this time. (electronic version- date received 12/03/2005 and fax version- date received 12/02/2005).
12/9/2005	DSCO Facsimile General Correspondence Response to FDA Request for Additional Information	Provided requested clinical information from the FDA's November 23, 2005 fax regarding the KL4-IRDS-02 and KL4-IRDS-06 clinical study reports and the SURFAXIN Integrated Summary of Efficacy. (3 Volumes)
1/4/2006	DSCO Facsimile General Correspondence Response to FDA Request for Additional Information	Provided notification of transfer of ownership of manufacturing facility.
1/20/2006	DSCO Facsimile General Correspondence	Provided additional information regarding the transfer of ownership of the SURFAXIN manufacturing facility .
1/20/2006	DSCO Letter General Correspondence	Provided additional information regarding the transfer of ownership of the SURFAXIN manufacturing facility.
2/7/2006	DSCO Letter General Correspondence	Provided "Coming Soon" promotional pieces (advertisement and CD-ROM) for SURFAXIN to DDMAC and the

Date	*Type of Submission*	*Description of Submission*
		Pulmonary Division.
3/2/2006	DSCO Letter General Correspondence	Provided updated draft vial and carton labels for SURFAXIN in response to the February 11, 2005 approvable letter.
3/31/2006	FDA Letter General Correspondence	Provided approvable letter for NDA 21-746 which includes deficiencies noted during the second review process.
3/31/2006	FDA Facsimile General Correspondence	Provided approvable letter for NDA 21-746 which includes deficiencies noted during the second review process.
4/6/2006	DSCO Letter General Correspondence	Notification of Intent to File an Amendment to the Approvable Letter Dated March 31, 2006.
5/23/2006	General Correspondence Response to Form FDA 483	Provided a copy of the May 19th submission to the North Brunswick, NJ District Office to the Form FDA 483 issued on April 7, 2006 for Discovery, Totowa to the Division. (copy of Form 483 included).
5/31/2006	General Correspondence Response to Form FDA 483	Provided a copy of the May 19th submission sent to the North Brunswick, NJ District Office in response to the Form FDA 483 issued on April 7, 2006 for Discovery, Totowa to the Parsippany Office.
9/1/2006	FDA Letter Establishment Inspection Report	Provided a copy of the Establishment Inspection Report (EIR) for the inspection conducted March 23, 2006 - April 7, 2006 at Discovery's Totowa facility.
9/27/2006	General Correspondence Meeting Request/Meeting Package	Request for a Type C meeting with the FDA to discuss CMC deficiencies identified in the March 31, 2006 Approvable Letter. Submission also serves as meeting package.
9/27/2006	DSCO Facsimile Meeting Request/Meeting Package	Provided a copy of the Type C meeting request/package for NDA 21-746 without attachments via facsimile.
10/6/2006	General Correspondence	Provided the FDA with Desk Copies of Type C Meeting Request/Meeting Package.
10/9/2006	General Correspondence	Provided the FDA with additional Desk Copies of Type C Meeting Request/Meeting Package.
10/16/2006	FDA Facsimile	Confirmation of December 21, 2006 Type C Meeting at 3:00 PM to discuss deficiencies noted in the FDA's March 31, 2006 letter.
11/16/2006	DSCO Facsimile	Provided supplement to September 27, 2006 meeting package (excluding two appendices) via fax which includes information regarding the qualification of the SURFAXIN drug product and drug substance impurities.
11/16/2006	General Correspondence Supplement to September 27, 2006 Meeting Package	Provided supplement to September 27, 2006 meeting package which includes information regarding the qualification of the SURFAXIN drug product and drug substance impurities.
11/27/2006	DSCO Facsimile Supplement to September 27, 2006 Meeting Package	Provided second supplement to September 27, 2006 meeting package via fax regarding the in-vivo bioassay and the production of new process validation batches.
11/27/2006	General Correspondence Supplement to September 27, 2006 Meeting Package	Provided second supplement to September 27, 2006 meeting package regarding the in-vivo bioassay and the production of new process validation batches.
12/14/2006	DSCO Facsimile General Correspondence	Provided revised list of meeting attendees for the December 21, 2006 meeting.
12/20/2006	FDA Facsimile	Comments for December 21, 2006 Meeting regarding questions contained in Discovery's submissions dated September 27, November 16 and November 27, 2006.
1/8/2007	General Correspondence Meeting Minutes	Provided a copy of Discovery's meeting minutes from the December 21, 2006 Meeting.
1/8/2007	DSCO Facsimile	Provided a copy of Discovery's meeting minutes from the

Date	*Type of Submission*	*Description of Submission*
	Meeting Minutes	December 21, 2006 Meeting via fax (Attachment not included in fax).
1/18/2007	FDA Facsimile Meeting Minutes	Agency's meeting minutes from the December 21, 2006 Meeting.
9/10/2007	General Correspondence Safety Updates	Request for FDA Feedback on Discovery's Proposed Extent and Format of the Safety Update Report.
10/22/2007	FDA Letter FDA Request for Additional Information	Agency's comments on Discovery's request sent 9/10/07 for the proposed extend and format of the requested safety update report (electronic version received 10/22/2007 and US mail version received 10/25/2007).
10/31/2007	Resubmission of NDA Application	Provided Discovery's complete response to the Agency's March 31, 2006 Approvable Letter (3 Sets of 16 volumes).
11/2/2007	Resubmission of NDA Application - Field Copy	Provided North Brunswick Field Office with a copy of Discovery's October 31, 2007 submission.
11/12/2007	Response to FDA Request for Additional Information	Provided draft labeling for resubmission dated October 31, 2007 to the Electronic Document Room.
11/15/2007	FDA Facsimile	FDA's acknowledgment of Discovery's October 31, 2007 resubmission, which is considered a complete, class 2 response to the March 31, 2006 Approvable Letter.
11/15/2007	FDA Letter	FDA's acknowledgment of Discovery's October 31, 2007 resubmission, which is considered a complete, class 2 response to the March 31, 2006 Approvable Letter. US Mail-date received 11/28/2007.
11/26/2007	General Correspondence	Provided additional desk copies of Discovery's resubmission dated October 31, 2007 (Module 1 & Response Items) to Regulatory Project Manager, at the FDA.
11/29/2007	FDA Request for Additional Information	FDA request for additional CMC information to continue the review of Discovery's 10/31/07 submission (electronic version- date received 11/29/2007 and US mail version- date received 12/05/2007).
11/30/2007	General Correspondence Response to FDA Observations from Form FDA 483 Dated September 24, 2007	Provided DSCO's response to the 483 dated 9/24/2007 to the Philadelphia District Office. Response includes 23 Exhibits.
12/5/2007	Response to FDA 483 Dated 9/24/07	Provided the Agency with a copy of the text portion of the submission package dated 11/30/07 to the FDA's Philadelphia District Office regarding the 483 dated 9/24/07.
12/7/2007	General Correspondence Response to FDA Request for Additional Information	Provided a written response to Agency's 11/29/2007 information request letter regarding closure dates of Totowa and date available for inspection, dates for stability data, and format of stability data.
12/10/2007	General Correspondence	Provided one additional desk copy of Discovery's resubmission dated 10/31/2007 (Module 1 & Response Items) to Regulatory Project Manager at the FDA.
12/20/2007	General Correspondence Response to FDA Request for Additional Information	Provided an update to Discovery's 12/7/2007 written response to the Agency's 11/29/2007 information request letter.
12/21/2007	General Correspondence Response to FDA Request for Additional Information	Provided a written response to Agency's 11/29/2007 information request letter.
1/4/2008	General Correspondence	Provided a copy of the draft labeling that was included in Discovery's 10/31/2007 in SPL format.
1/4/2008	General Correspondence	Provided NJ District Field Office with a copy of Discovery's 12/21/2007 submission to the Division regarding the Agency's

Date	*Type of Submission*	*Description of Submission*
		11/29/2007 information request letter.
1/8/2008	General Correspondence (E-mail) Response to FDA Request for Additional Information	Provided response to LA District Field Office's 1/8/08 request regarding testing facility. Information included: background information on SURFAXIN and all applicable sections from Discovery's 10/31/07 response which refer to testing.
1/11/2008	FDA Letter Request for Additional Information	FDA Request for an updated list of all sites involved in the manufacturing and testing of the drug product and drug substances, including activities performed, and contact information. For sites no longer involved date of last involvement and whether or not the site should remain active as an alternate site.
1/18/2008	General Correspondence Response to FDA Request for Additional Information	Provided a written response to the Agency's 1/11/2008 request. Items included: updated list of all sites currently involved in the manufacturing and testing of the drug product and drug substances and replacement pages for sections 3.2.S.2.1 and 3.2.P.3.1 (Manufacturers) of Module 3 to reflect the updated list of manufacturing and testing sites.
1/21/2008	General Correspondence Response to FDA Request for Additional Information	Provided response to Wilmington DE office 1/18/2008 request regarding testing facility. Provided information via e-mail. Information included: Sections 3.2.P.3.1, 3.2.P.2.5., 3.2.P.8.2, and 3.2.P.5.1 from October 31, 2007 complete response.
1/25/2008	General Correspondence Response to FDA Request for Additional Information	Provided the NJ District Office with a copy of DSCO's 1/18/2008 response to the Agency's 1/11/2008 information request letter. Items included: Updated list of all sites currently involved in the manufacturing and testing of the drug product and drug substances and replacement pages for sections 3.2.S.2.1 and 3.2.P.3.1 (Manufacturers) of Module 3 to reflect the updated list of manufacturing and testing sites.
2/29/2008	General Correspondence Response to FDA Request for Additional Information	Provided the Agency with stability data on Surfaxin new process validation lots.
3/3/2008	General Correspondence Response to FDA Request for Additional Information	Provided the NJ District Office with a coy of DSCO's 2/29/08 submission that included stability data on Surfaxin new process validation lots.
4/7/2008	General Correspondence Response to FDA Observations from Form FDA 483 Dated 3/25/2008	Provided the Parsippay, NJ District Office with Discovery's response to the Form FDA 483 Dated March 25, 2008. (Totowa SOPs provided as attachments).
4/10/2008	General Correspondence Response to FDA Observations from Form FDA 483 Dated 3/25/2008	Provided the Division with a copy of Discovery's 4/7/2008 response to the Parsippay, NJ District Office regarding the Form FDA 483 Dated 3/25/2008. (Totowa SOPs provided as attachments).
4/14/2008	FDA Facsimile (Sent via Email)	FDA Comments regarding DSCO's draft labeling submitted in DSCO's resubmission dated 10/31/2007. (Submitted to Electronic Document Room 11/12/2007).
4/21/2008	General Correspondence	DSCO Response to FDA Labeling Comments Dated 4/14/2008. Attachments include draft PI.
4/25/2008	FDA Facsimile (Sent via Email)	FDA Comments regarding DSCO's draft labeling (PI) submitted 4/21/2008.
4/29/2008	General Correspondence Response to FDA Request for Additional Information	Provided the Agency with DSCO's revised draft package insert dated 4/28/08 in response to the Agency's 4/25/08 fax.
4/29/2008	General Correspondence Response to FDA Request for	Provided the Agency with DSCO's revised draft vial and carton labels in response to the Agency's 4/14/08 comments.

Date	*Type of Submission*	*Description of Submission*
	Additional Information	
4/29/2008	General Correspondence Response to FDA Request for Additional Information	Provided the Agency with information regarding activities/responsibilities that were transferred from Laureate Princeton, NJ to Discovery.
5/1/2008	FDA Letter	FDA's Approvable Letter (18 CMC Comments and 2 Draft Labeling Comments) (electronic version dated 5/1/2008, US mail date received 05/06/2008).
5/5/2008	General Correspondence	Notification of Intent to File Amendment to the Approvable Letter Dated 5/1/2008.
5/14/2008	General Correspondence	End of Review Meeting Request/Package- Regarding Approvable Letter Dated 5/1/2008.
5/20/2008	General Correspondence	Desk Copies for June 18, 2008 End-of Review Meeting
5/28/2008	General Correspondence	FDA confirmation of End- of-Review meeting scheduled for 06/18/2008.
6/3/2008	General Correspondence	Provided the Agency with 12 desk copies of June 3, 2008 Meeting Submission (Supplement to the May 14, 2008 End of Review Meeting Package).
6/3/2008	General Correspondence	Provided the Agency with 3 copies of Meeting Submission-Supplement to the May 14, 2008 End of Review Meeting Package.
6/17/2008	FDA Facsimile (Sent via Email)	FDA comments regarding DSCO's questions submitted in the May 14, 2008 Meeting Package Submission
7/14/2008	FDA Meeting Minutes	FDA Meeting Minutes from June 18, 2008 Teleconference
10/17/2008	Resubmission of NDA Application	Provided Discovery's complete response to the Agency's May 1, 2008 Approvable Letter (Total of 7 volumes). Response consisted of the following: Response Items (1 volumes), Module 1 (1 volume), Module 3 (2 volumes), and Method Validation Package (3 volumes).
10/17/2008	General Correspondence	Stamped Received Cover Letters for Resubmission dated 17October2008 from the Division, Division EDR (includes CD ROM) and NJ Field Office.
11/7/2008	FDA Letter	FDA response to the 10/17/2008 resubmission. The response is considered a Class 2 response (electronic version). Letter received via US mail received 11/20/2008.
11/25/2008	General Correspondence	Provided 4 additional copies of Resubmission dated 10/17/2008- Response Items (1 Vol.), Mod 1 (Vol 1) and Mod 3 (Vol 1 and 2).
2/12/2009	General Correspondence	Tightening of Surfaxin Drug Product Acceptance Criteria (Revised Proposed Limits for Lipid-Related Impurities DG2 and DPPA)
2/17/2009	General Correspondence	Field Copy of Minor Amendment sent on 02/12/2009 regarding; Tightening of Surfaxin Drug Product Acceptance Criteria.
3/12/2009	General Correspondence	Response to March 4, 2009 Teleconference.
3/13/2009	Response to FDA Request for Additional Information	1 Desk Copy of April 7,2008 submission to the NJ District Office
4/17/2009	FDA Complete Response Letter	FDA Complete Response Letter.
4/24/2009	General Correspondence End-of-Review Meeting Request/Package	End-of-Review Meeting Request/Package to discuss FDA's comments regarding the in-vivo BAT and Discovery's reliance on literature from the April 17, 2009 Complete Response Letter.
5/11/2009	End-of-Review Meeting Request Grant Letter	FDA's End-of-Review meeting request grant letter.
5/19/2009	General Correspondence	Supplement to April 24, 2009 End-of-Review Meeting

Date	*Type of Submission*	*Description of Submission*
	Supplement to April 24, 2009 End-of-Review Meeting Request/Package	Request/Package.
5/21/2009	General Correspondence	Request to expunge March 27, 2009 letter.
6/1/2009	General Correspondence	FDA Responses to Questions in Briefing Package (April 24, 2009 original request and May 19, 2009 supplement) for June 2, 2009 meeting.
6/16/2009	General Correspondence FDA Meeting Minutes	FDA Meeting Minutes from June 2, 2009 Meeting (Received via US Mail on 6/23/2009)
6/26/2009	General Correspondence	Proposed revisions to the FDA's meeting minutes dated June 16, 2009 from the June 2, 2009 meeting.
8/5/2009	General Correspondence Type C Meeting Request/Briefing Package	Meeting Request/Package to discuss the design of a limited clinical trial.
9/2/2009	General Correspondence FDA Meeting Grant Letter	FDA Meeting Grant Letter in response to August 5, 2009 meeting request. Type C Meeting Date September 29, 2009 (Received via e-mail 9/2 and US Mail 9/9)
9/2/2009	General Correspondence Additional Copies of August 5, 2009 Type C Meeting Request/Package	Provided 3 additional copies of August 5, 2009 Type C Meeting Request/Package.
9/11/2009	General Correspondence Updated Discovery Meeting Attendee List	Provided updated Discovery attendee list for September 29, 2009 teleconference.

§1.740(a)(12): Eligibility For, and Length Of, Interim Extension of Patent Term

Discovery Labs believes that U.S. Patent No. 5,789,381 is eligible for interim extension under 35 U.S.C. §156 because it satisfies all the requirements for such extension as follows:

35 U.S.C. §156(a). The '381 patent claims a drug product and a method of using a drug product.

35 U.S.C. §156(a)(1). The term of the '381 patent has not expired before submission of the instant application.

35 U.S.C. §156(a)(2). The term of the '381 patent has never been extended under 35 U.S.C. §156(e)(1).

35 U.S.C. §156(a)(3). The instant application is being submitted by the agent (Discovery Labs) of the owner of record (Scripps) of the '381 patent, and in accordance with the requirements of 35 U.S.C. §156(d) and the rules of the U.S. Patent and Trademark Office.

35 U.S.C. §156(a)(4). The pending product for which approval is sought, Surfaxin®, and its method of use, have been subject to a regulatory review period before commercial marketing or use.

35 U.S.C. §156(a)(5)(A). The commercial marketing or use of the pending product, Surfaxin®, after the regulatory review period, will be the first permitted commercial marketing or use of the pending product under the provisions of the FFDCA. (21 U.S.C. §355), under which such regulatory review period will occur.

35 U.S.C. §156(c)(4). No other patent has been extended under 35 U.S.C. §156(e)(1) for the same regulatory review period as the pending product Surfaxin®. Discovery Labs is filing concurrently with this application, additional initial applications for interim extensions of patent term under 35 U.S.C. §156(d)(5) for U.S. Patent No. 5,260,273 and U.S. Patent No. 5,407,914, which are subject to the same regulatory review period for the pending product Surfaxin®. Because these applications are for ***interim*** extensions under 35 U.S.C. §156(d)(5), they do not qualify as patents extended under 35 U.S.C. §156(e)(1) for the same regulatory review period for the pending product Surfaxin®. Accordingly, the present application is eligible for interim patent term extension under 35 U.S.C. §156(c)(4).

The length of interim extension of the term of the '381 patent claimed by Discovery Labs is one (1) year.

The maximum length of term extension available for the '381 patent based on approval of Surfaxin®, when approved, will be five years, and the length of extension will be determined as follows:

As defined in 37 CFR §1.775(c), the length of the regulatory review period for a human drug is the sum of (1) and (2) below:

> (1) The number of days in the period beginning on the date an exemption under subsection (i) of §505 or subsection (d) of §507 of the FFDCA became effective for the approved product and ending on the date the application was initially submitted for such product under those sections and
>
> (2) The number of days in the period beginning on the date the application was initially submitted for the approved product under subsection (b) of §505 or §507 of the FFDCA and ending on the date the application was approved.

The period specified by (1) is the number of days in the period beginning on the date IND No. 40,287 became effective, *i.e.*, Sept. 5, 1992, and ending on the date NDA No. 21-746 was initially submitted, *i.e.*, April 13, 2004, which is 4239 days. The regulatory review period under (2) is still ongoing but has lasted over 1982 days as of September 15, 2009. Accordingly, as of September 15, 2009, the length of the regulatory review period is 4239 days plus 1982 days, or 6221 days.

Pursuant to 37 CFR §1.775(d), Discovery Labs expects that the length of the extension available for the '381 patent will be the maximum allowed, which is five years. The term of the '381 patent as extended was calculated as follows:

> **37 CFR §1.775(d)(1)(i)**. The '381 patent issued on Aug. 4, 1998. The number of days in periods (1) and (2) above that were on and before the date on which the patent issued, all in period (1), is 2160 days. The number of days in period (1), above, is 4239 days. The period specified by 37 CFR §1.775(d)(1)(i) is therefore 2079 days.
>
> **37 CFR §1.775(d)(1)(ii)**. Applicant has acted with due diligence during the entire regulatory review period. Accordingly, the period specified by 37 CFR §1.775(d)(1)(ii) is 0 days.
>
> **37 CFR §1.775(d)(1)(iii)**. The period specified by 37 CFR §1.775(d)(1)(i) is 2079 days. One half of this period is 1039.5 days (treated as 1039 days). The period specified by 37 CFR §1.775(d)(1)(iii) is therefore 3103 days (6221 - 2079 - 1039).
>
> **37 CFR §1.775(d)(2)**. The original term of the '381 patent, as shortened by terminal disclaimers, expires Nov. 17, 2009. Adding 3103 days to this term would extend the term of the '381 patent to May 17, 2018.
>
> **37 CFR §1.775(d)(3).** NDA No. 21-746 has not yet been approved. If the NDA were approved on Sept. 15, 2009, the date specified by 37 CFR §1.775(d)(3) would be Sept. 15, 2023. Accordingly, the date specified by 37 CFR §1.775(d)(3) can be no earlier than Sept. 15, 2023.

37 CFR §1.775(d)(4). Since the date specified by 37 CFR §1.775(d)(3) can be no earlier than Sept. 15, 2023, the earlier of this date and the date specified by 37 CFR §1.775(d)(2) is May 17, 2018.

37 CFR §1.775(d)(5). The original term of the '381 patent expires on Nov. 17, 2009. Adding five years to that date results in a date of Nov. 17, 2014. The earlier of this date and the date specified by 37 CFR §1.775(d)(4) is Nov. 17, 2014.

Accordingly, pursuant to 37 CFR §1.755(d), Discovery Labs expects that the length of the extension available for the '381 patent will be the maximum allowed, which is five years. Discovery Labs also reasonably expects that the applicable regulatory review period under 35 U.S.C. §156(g)(1)(B) that began for Surfaxin® will extend beyond Nov. 17, 2009, the date of expiration of the '381 patent. Discovery Labs therefore requests a first interim one year extension of the term of U.S. Patent No. 5,789,381.

§1.740(a)(13): Duty of Disclosure

Discovery Labs acknowledges a duty to disclose to the Director of the U.S. Patent and Trademark Office and to the Secretary of Health and Human Services any information which is material to the determination of entitlement to the extension sought herein.

§1.740(a)(14): Fees

Pursuant to 37 CFR §1.20(j)(3), the Commissioner is hereby authorized to charge any fees which may be required by the accompanying papers, or credit any overpayment to deposit Account No. **50-2778** (Our Order No. 382615 (105264)).

§1.740(a)(15): Name and Address for Correspondence

Please direct all inquiries and correspondence relating to this matter to:

Dechert LLP
c/o Ann M. Caviani Pease
2440 W. El Camino Real, Suite 700
Mountain View, CA 94040-1499
Tel 1-650-813-4800

§1.740(b)

Two additional copies of this application are included herewith (for a total of three copies).

Declaration

The undersigned duly authorized representative of Discovery Labs hereby declares:

(a) that he is a patent attorney authorized to practice under the United States Patent and Trademark Office and has power of attorney from Discovery Labs for the purpose of transacting all matters related to U.S. Patent 5,789,381. Discovery Labs has agency power from its exclusive licensor, Scripps, to conduct matters relating to extension of U.S. Patent 5,789,381 before the U.S. Patent and Trademark Office and to select its choice of counsel. Documents evidencing this are attached as **Exhibits A** and **J**.

(b) that he has reviewed and understands the contents of this application being submitted pursuant to 35 U.S.C. §156(d)(5); and

(c) that he believes that the patent is entitled to interim extension pursuant to 37 CFR §1.790.

Respectfully submitted,

DECHERT LLP

Date: October 6, 2009

George A. Senich

Registration No. 42,140
Attorney for Applicant, Discovery Labs

DECHERT LLP
Customer No. 37509
Tel: 650.813.4800
Fax: 650.813.4848

Attached concurrently herewith are the following documents in support of the present application:

Exhibit A:	Appointment of Agent by Assignee;
Exhibit B:	Prior Corporate Names of Discovery Labs;
Exhibit C:	Identification of Product;
Exhibit D:	Copy of U.S. Patent No. 5,789,381;
Exhibit E:	Patent Bibliographic Data;
Exhibit F:	Certificate(s) of Correction of U.S. Patent No. 5,789,381;
Exhibit G:	Terminal Disclaimer(s) of U.S. Patent No. 5,789,381;
Exhibit H:	U.S. IND No. 40,287 Index;
Exhibit I:	U.S. NDA No. 21-746 Index; and
Exhibit J:	Power of Attorney

Exhibit A

THE
SCRIPPS
RESEARCH
INSTITUTE®

Thomas Fitting, Esq., Ph.D.
Chief Patent Counsel

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e-mail: fitting@scripps.edu

September 3, 2009

David L. Lopez, Esq.,
Executive Vice President, General Counsel
Discovery Laboratories, Inc.
2600 Kelly Road, Suite 100
Warrington, PA 18976-3622

RE: Application for Extension of Term for U.S. Patent Nos. 5,260,273, 5,407,914 and 5,789,381 Under 35 U.S.C. Section 156

Dear Mr. Lopez:

This letter confirms that the Scripps Research Institute ("Scripps") is the assignee of the entire right, title and interest, of U.S. Patent Nos. 5,260,273, 5,407,914, and 5,789,381, in each case subject to the confirmatory license to U.S. Government, as evidenced by the assignments recorded in the U.S. Patent and Trademark Office ("USPTO") as follows:

Patent No.	Issue Date	Reel No.	Frame No.
5,260,273	November 9, 1993	005808	0191
5,407,914	April 18, 1995	015167	0725
5,789,381	August 4, 1998	015215	0518

By this letter, Scripps revokes all previously appointed entities and individuals and appoints Discovery Laboratories, Inc. ("Discovery Labs") as its sole agent to prosecute the Application for Extension of U.S. Patent Nos. 5,260,273, 5,407,914, and 5,789,381 under 35 U.S.C. Section 156 and to handle all matters regarding the above patents. Included herein is the authority of Discovery Labs to grant power to Discovery Labs counsel of choice to transact all matters before the USPTO.

Kind Regards,

The Scripps Research Institute

Thomas Fitting, Ph.D., Esq.,
Chief Patent Counsel

Exhibit B

STATE OF DELAWARE
CERTIFICATE OF OWNERSHIP

SUBSIDIARY INTO PARENT
Section 253

CERTIFICATE OF OWNERSHIP
MERGING

ATI ACQUISITION CORP.

INTO

DISCOVERY LABORATORIES, INC.

Pursuant to Section 253 of the General Corporation Law
of the State of Delaware

Discovery Laboratories, Inc., a corporation originally incorporated under the name of Ansan, Inc. on the 6th day of November, 1992 and subsequently changed to Discovery Laboratories, Inc. on the 25th day of November, 1997 (the "Corporation"), pursuant to the provisions of the General Corporation Law of the State of Delaware:

DOES HEREBY CERTIFY that the Corporation owns 100% of the outstanding shares of each class of stock of ATI Acquisition Corp., a corporation originally incorporated under the name of Acute Therapeutics, Inc. on the 11th day of September, 1996, and amended its name to ATI Acquisition Corp. on the 16th day of June, 1998, pursuant to the provisions of the General Corporation Law of the State of Delaware ("ATI") and that the Corporation, by a resolution of its Board of Directors duly adopted at a meeting held on the 16th day of February, 1999, determined to and did merge ATI into itself, which resolution is as follows:

WHEREAS the Corporation lawfully owns 100% of the outstanding shares of each class of stock of ATI, a corporation organized and existing under the laws of the State of Delaware; and

WHEREAS in the judgment of this Board of Directors it is desirable for business reasons to merge ATI into the Corporation and to be possessed of all the estate, property, rights, and privileges of ATI;

NOW THEREFORE, upon motion duly made, seconded and carried, it was unanimously

STATE OF DELAWARE
SECRETARY OF STATE
DIVISION OF CORPORATIONS
FILED 12:05 PM 10/25/1999
991450428 – 2315242

RESOLVED, that such merger be effected by transferring all the assets and related liabilities of ATI into the Corporation; and

FURTHER RESOLVED, that an authorized officer of the Corporation be and is hereby directed to make and execute a certificate of ownership setting forth a copy of resolution to so merge and assume ATI's liabilities and obligations, the date of adoption thereof, and to file the same in the office of the Secretary of the State of Delaware; and

FURTHER RESOLVED, that the officers of the Corporation be, and they hereby are, authorized, empowered, and directed to do and perform all such further acts and things, to execute and deliver in the name of the Corporation, and where necessary or appropriate, to file with the appropriate governmental authorities, all such further certificates, instruments, or other documents, as in their judgment shall be necessary or advisable in order to effectuate such merger, the intent and purposes of the foregoing resolutions, and any or all of the transactions contemplated therein.

IN WITNESS WHEREOF, Discovery Laboratories, Inc., for the purpose of merging ATI into Discovery Laboratories, Inc. under the laws of the State of Delaware, has caused this Certificate of Ownership to be executed in its corporate name this 21st day of October, A.D. 1999.

By: _____
Authorized Officer

Name: Robert J. Capetola

Title: President / CEO

Exhibit C

1.1 Descriptive Information

Surfaxin®, generically known by the name lucinactant, and having CAS Reg. No. 825600-90-6, comprises the following active ingredients:

(1) Sinapultide (KL_4 peptide);
(2) colfosceril palmitate (dipalmitoylphosphatidylcholine [DPPC]);
(3) palmitoyloleoylphosphatidyl glycerol, sodium salt (POPG); and
(4) palmitic acid (PA).

1.2 Active Pharmaceutical Ingredient: Sinapultide

Chemical Name:

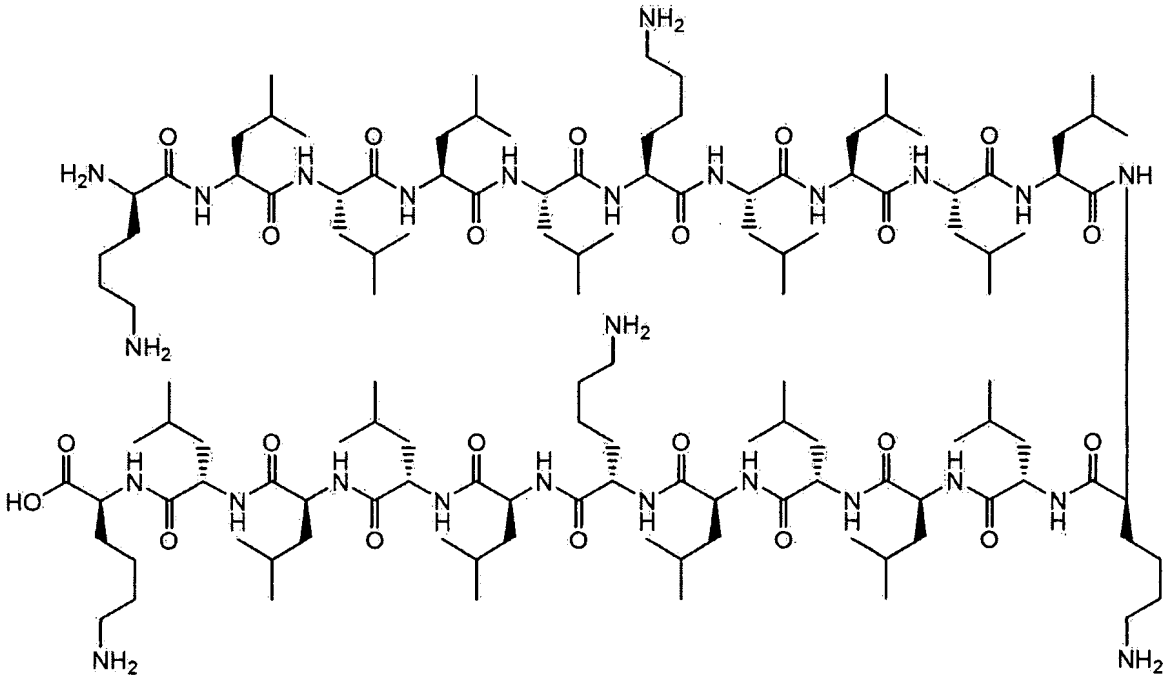
L-Lysyl-L-leucyl-L-leucyl-L-leucyl-L-leucyl-L-lysyl-L-leucyl-L-leucyl-L-leucyl-L-leucyl-L-lysyl-L-leucyl-L-leucyl-L-leucyl-L-leucyl-L-lysyl-L-leucyl-L-leucyl-L-leucyl-L-leucyl-L-lysine

Nonproprietary Name: Sinapultide (USAN; INN)

Other Designations: KL_4

CAS Number: 138531-07-4

Chemical Structure:



Molecular Formula: $C_{126}H_{238}N_{26}O_{22}$

Molecular Weight: 2469.4

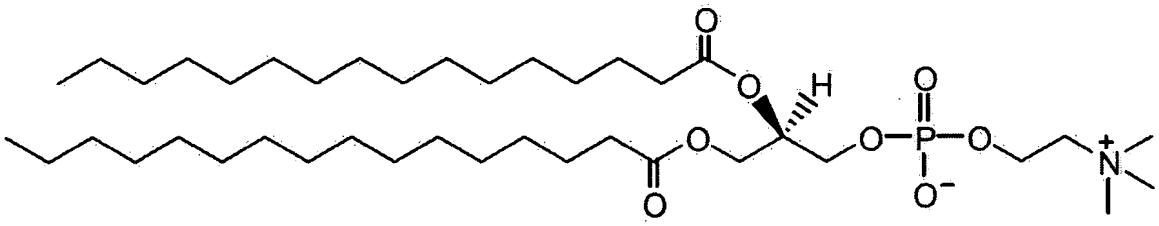
1.3 Active Pharmaceutical Ingredient: Colfosceril Palmitate [DPPC]

Chemical Names: (1) 3,5,9-Trioxa-4-phosphapentaconsa-1-aminium, 4-hydroxy-*N,N,N*-trimethyl-1-oxo-7-[(1-oxohexadecyl)- oxy]-, hydroxide, inner salt, 4-oxide, (*R*)-; (2) Choline hydroxide, dihydrogen phosphate, inner salt, ester with L-1,2-dipalmitin; (3) 1,2-Dipalmitoyl-*sn*-Glycero-3-Phosphocholine

Nonproprietary Name: Colfosceril Palmitate (USAN; INN; BAN)
Other Designations: DPPC

CAS Number: 63-89-8

Chemical Structure:



Molecular Formula: $C_{40}H_{80}NO_8P$

Molecular Weight: 734.05

1.4 Active Pharmaceutical Ingredient: Palmitoyl-2-Oleoyl-3-[Phospho-rac-(1-Glycerol)] Sodium Salt [POPG]

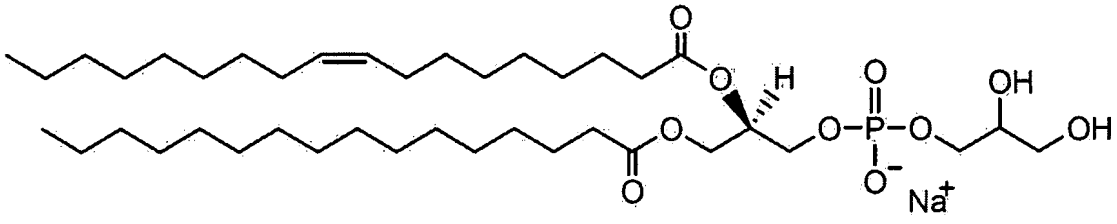
Chemical Name: 1-Palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoglycerol

Nonproprietary Name: None

Other Designations: POPG; L-α-palmitoyl-oleoyl phosphatidylglycerol

CAS Number: 13879-80-6

Chemical Structure:



Molecular Formula: $C_{40}H_{76}PO_{10}Na$

Molecular Weight: 771.01

1.5 Active Pharmaceutical Ingredient: Palmitic Acid

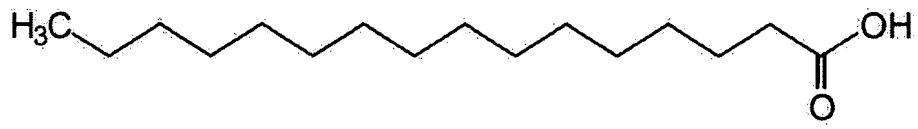
Chemical Name: Hexadecanoic acid

Nonproprietary Name: Palmitic Acid (USAN; INCI)

Other Designations: Hexadecylic acid; cetylic acid

CAS Number: 57-10-3

Chemical Structure:



Molecular Formula: $C_{16}H_{32}O_2$

Molecular Weight: 256.43

Exhibit D

US005789381A

United States Patent [19]

Cochrane et al.

[11] Patent Number: 5,789,381

[45] Date of Patent: Aug. 4, 1998

[54] **PULMONARY SURFACTANT PROTEINS AND RELATED POLYPEPTIDES**

[75] Inventors: **Charles G. Cochrane**, La Jolla; **Susan D. Revak**, San Diego, both of Calif.

[73] Assignee: **The Scripps Research Institute**, La Jolla, Calif.

[21] Appl. No.: **419,824**

[22] Filed: **Apr. 11, 1995**

Related U.S. Application Data

[63] Continuation of Ser. No. 60,833, May 12, 1993, Pat. No. 5,407,914, which is a continuation-in-part of Ser. No. 715,397, Jun. 14, 1991, Pat. No. 5,260,273, which is a continuation-in-part of Ser. No. 293,201, Jan. 4, 1989, Pat. No. 5,164,369, which is a continuation-in-part of Ser. No. 141,200, Jan. 6, 1988, abandoned.

[51] **Int. Cl.**[6] **A61K 38/16**

[52] **U.S. Cl.** **514/13**; 424/450; 530/326

[58] **Field of Search** 514/2, 15, 14, 514/13, 12; 530/324, 326, 327, 328; 424/450

[56] **References Cited**

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5,260,273	11/1993	Cochrane et al.	514/12
5,407,914	4/1995	Cochrane et al.	514/12

Primary Examiner—Cecilia J. Tsang
Assistant Examiner—Patrick R. Delaney
Attorney, Agent, or Firm—Olson & Hierl, Ltd.

[57] **ABSTRACT**

The present invention discloses useful peptides and synthetic pulmonary surfactants, as well as methods of making and using same. In one preferred embodiment, a synthetic pulmonary surfactant comprises one or more pharmaceutically acceptable phospholipids admixed with a polypeptide comprising at least 10 amino acid residues and no more than about 60 amino acid residues, said polypeptide including a sequence having alternating hydrophobic and hydrophilic amino acid residue regions. In other embodiments, a surfactant peptide has an amino acid residue sequence selected from the group consisting of KLLLLKLLLLKLLLLKLLLLK, KLLLLLLLLKLLLLLLLLKLL, and KKLLLLLLLKKLLLLLLLKKL.

6 Claims, 8 Drawing Sheets

```
CAC CTG GGC CTG TGC AAA TCC CGG CAG CCA GAG CCA GAG CAG GAG        45
His Leu Gly Leu Cys Lys Ser Arg Gln Pro Glu Pro Glu Gln Glu
-62

CCA GGG ATG TCA GAC CCC CTG CCC AAA CCT CTG CGG GAC CCT CTG        90
Pro Gly Met Ser Asp Pro Leu Pro Lys Pro Leu Arg Asp Pro Leu

CCA GAC CCT CTG CTG GAC AAG CTC GTC GTC CCT GTG CTG CCC GGG       135
Pro Asp Pro Leu Leu Asp Lys Leu Val Val Pro Val Leu Pro Gly

GCC CTC CAG GCG AGG CCT GGG CCT CAC ACA CAG GAT CTC TCC GAG       180
Ala Leu Gln Ala Arg Pro Gly Pro His Thr Gln Asp Leu Ser Glu

CAG CAA TTC CCC ATT CCT CTC CCC TAT TGC TGG CTC TGC AGG GCT       225
Gln Gln Phe Pro Ile Pro Leu Pro Tyr Cys Trp Leu Cys Arg Ala
    -1  1

CTG ATC AAG CGG ATC CAA GCC ATG ATT CCC AAG GGT GCG CTA GCT       270
Leu Ile Lys Arg Ile Gln Ala Met Ile Pro Lys Gly Ala Leu Ala

GTG GCA GTG GCC CAG GTG TGC CGC GTG GTA CCT CTG GTG GCG GGC       315
Val Ala Val Ala Gln Val Cys Arg Val Val Pro Leu Val Ala Gly

GGC ATC TGC CAG TGC CTG GCT GAG CGC TAC TCC GTC ATC CTG CTC       360
Gly Ile Cys Gln Cys Leu Ala Glu Arg Tyr Ser Val Ile Leu Leu

GAC ACG CTG CTG GGC CGC ATG CTG CCC CAG CTG GTC TGC CGC CTC       405
Asp Thr Leu Leu Gly Arg Met Leu Pro Gln Leu Val Cys Arg Leu

GTC CTC CGG TGC TCC ATG GAT GAC AGC GCT GGC CCA AGG TCG CCG       450
Val Leu Arg Cys Ser Met Asp Asp Ser Ala Gly Pro Arg Ser Pro
                          81

ACA GGA GAA TGG CTG CCG CGA GAC TCT GAG TGC CAC CTC TGC ATG       495
Thr Gly Glu Trp Leu Pro Arg Asp Ser Glu Cys His Leu Cys Met

TCC GTG ACC ACC CAG GCC GGG AAC AGC AGC GAG CAG GCC ATA CCA       540
Ser Val Thr Thr Gln Ala Gly Asn Ser Ser Glu Gln Ala Ile Pro
                          110

CAG GCA ATG CTC CAG GCC TGT GTT GGC TCC TGG CTG GAC AGG GAA       585
Gln Ala Met Leu Gln Ala Cys Val Gly Ser Trp Leu Asp Arg Glu

AAG TGC AAG CAA TTT GTG GAG CAG CAC ACG CCC CAG CTG CTG ACC       630
Lys Cys Lys Gln Phe Val Glu Gln His Thr Pro Gln Leu Leu Thr

CTG GTG CCC AGG GGC TGG GAT GCC CAC ACC ACC TGC CAG GCC CTC       675
Leu Val Pro Arg Gly Trp Asp Ala His Thr Thr Cys Gln Ala Leu

GGA GTG TGT GGG ACC ATG TCC AGC CCT CTC CAG TGT ATC CAC AGC       720
Gly Val Cys Gly Thr Met Ser Ser Pro Leu Gln Cys Ile His Ser

CCC GAC CTT TGATGAGAAC TCAGCTGTCCA                                750
Pro Asp Leu
          181
```

FIG. 1

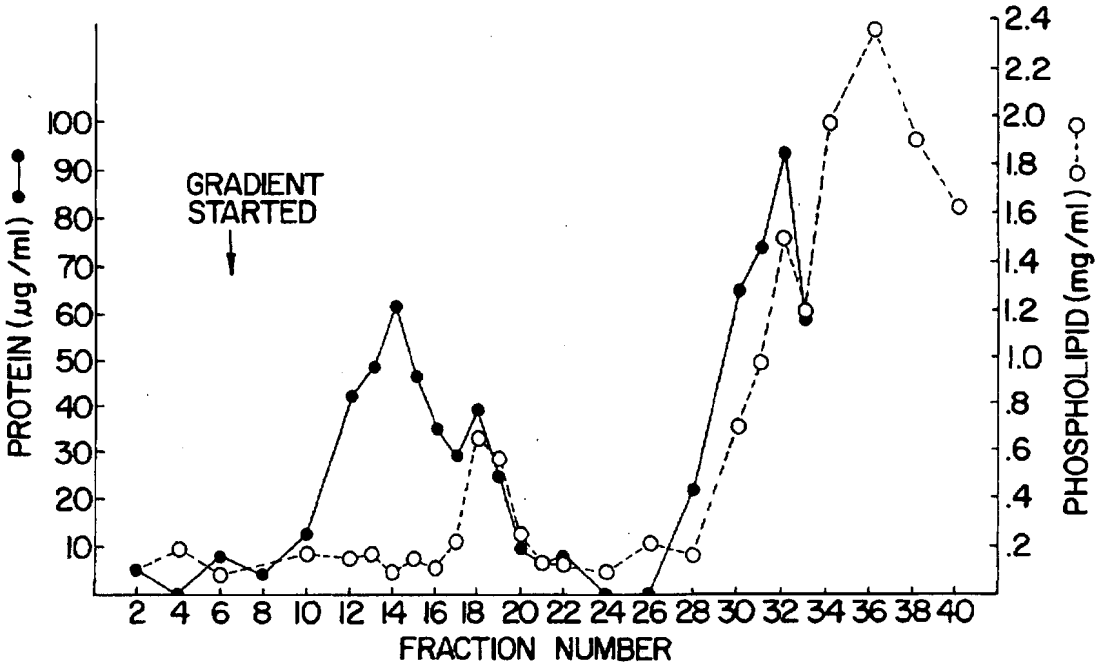
PROTEIN (μg/ml)
PHOSPHOLIPID (mg/ml)
GRADIENT STARTED
FRACTION NUMBER
100
90
80
70
60
50
40
30
20
10
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
.8
.6
.4
.2
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40

FIG.2

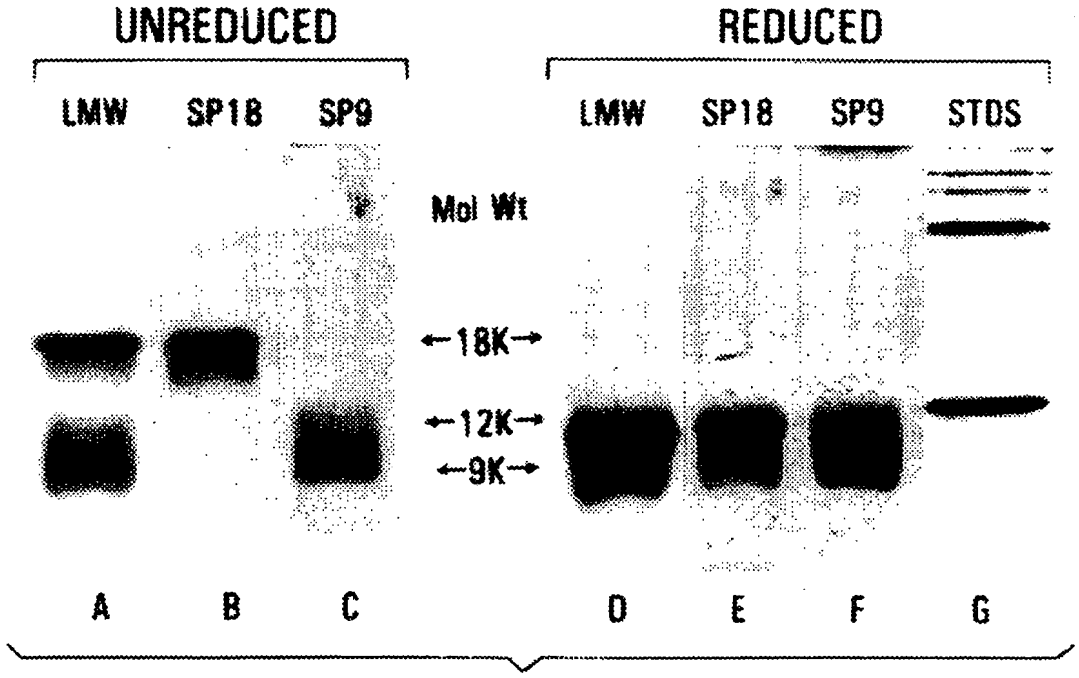
UNREDUCED
REDUCED
LMW
SP18
SP9
LMW
SP18
SP9
STDS
Mol Wt
←18K→
←12K→
←9K→
A
B
C
D
E
F
G

FIG.3

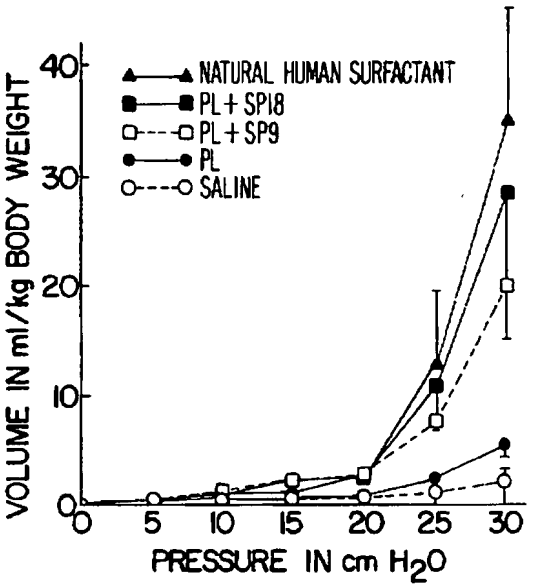
NATURAL HUMAN SURFACTANT
PL + SP18
PL + SP9
PL
SALINE
VOLUME IN ml/kg BODY WEIGHT
PRESSURE IN cm H_2O
0
5
10
15
20
25
30
40

FIG. 4A

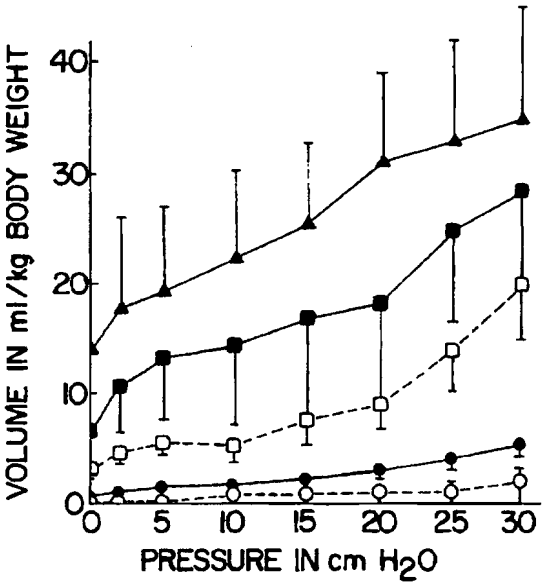
VOLUME IN ml/kg BODY WEIGHT
PRESSURE IN cm H_2O
0
5
10
15
20
25
30
40

FIG. 4B

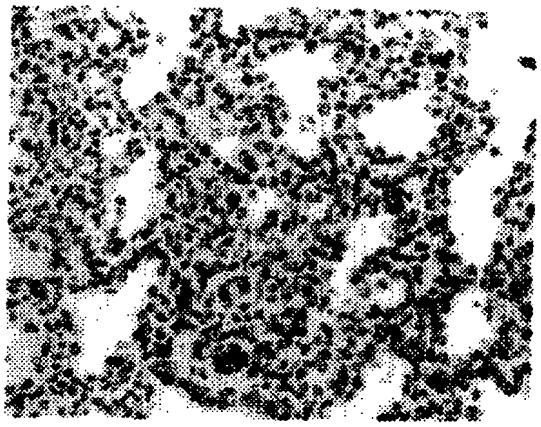

FIG.5A

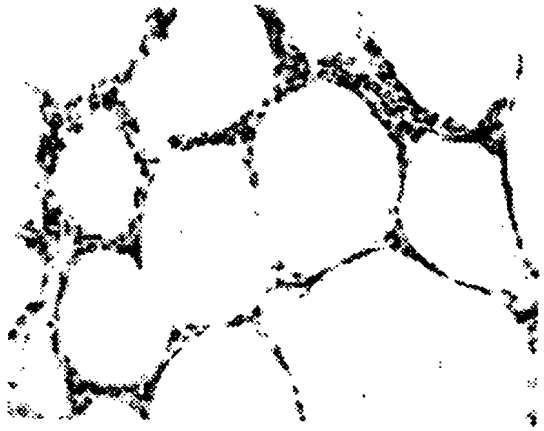

FIG.5B

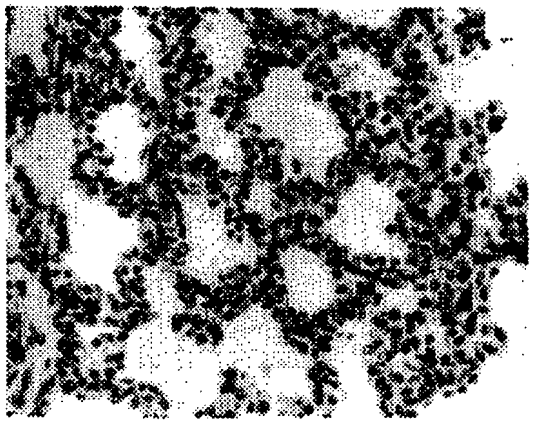

FIG.5C

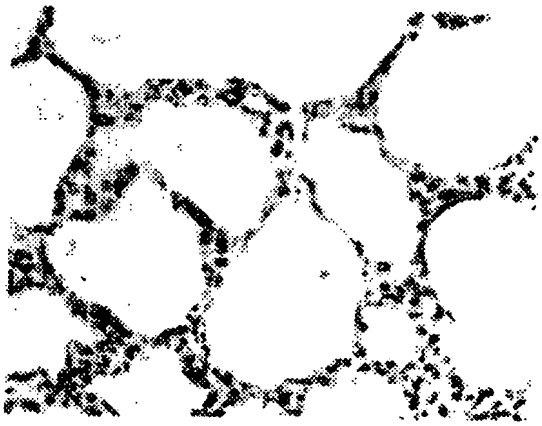

FIG.5D

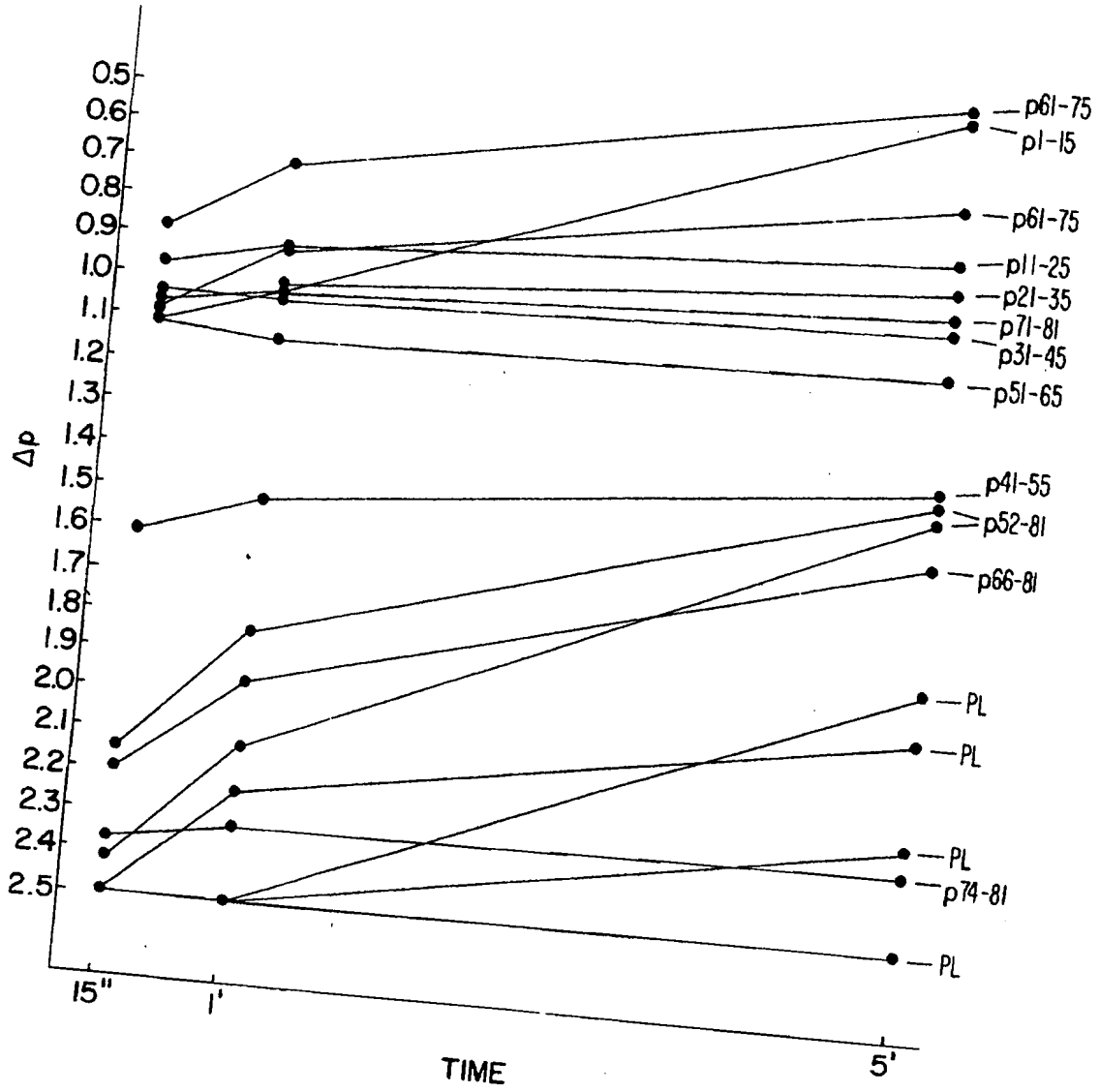
0.5
0.6
0.7
0.8
0.9
1.0
1.1
1.2
1.3
1.4
1.5
1.6
1.7
1.8
1.9
2.0
2.1
2.2
2.3
2.4
2.5
Δρ
15"
1'
5'
TIME
p61-75
p1-15
p61-75
p11-25
p21-35
p71-81
p31-45
p51-65
p41-55
p52-81
p66-81
PL
PL
PL
p74-81
PL

FIG.6

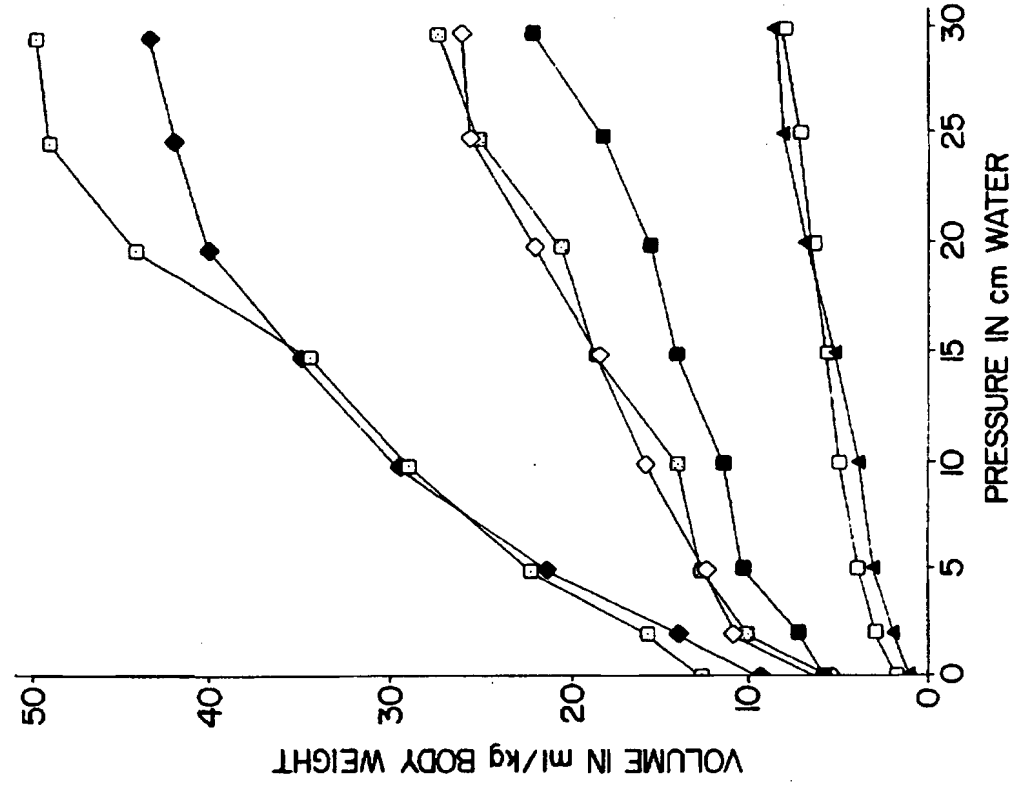
VOLUME IN ml/kg BODY WEIGHT
0
10
20
30
40
50
PRESSURE IN cm WATER
0
5
10
15
20
25
30

FIG. 7B

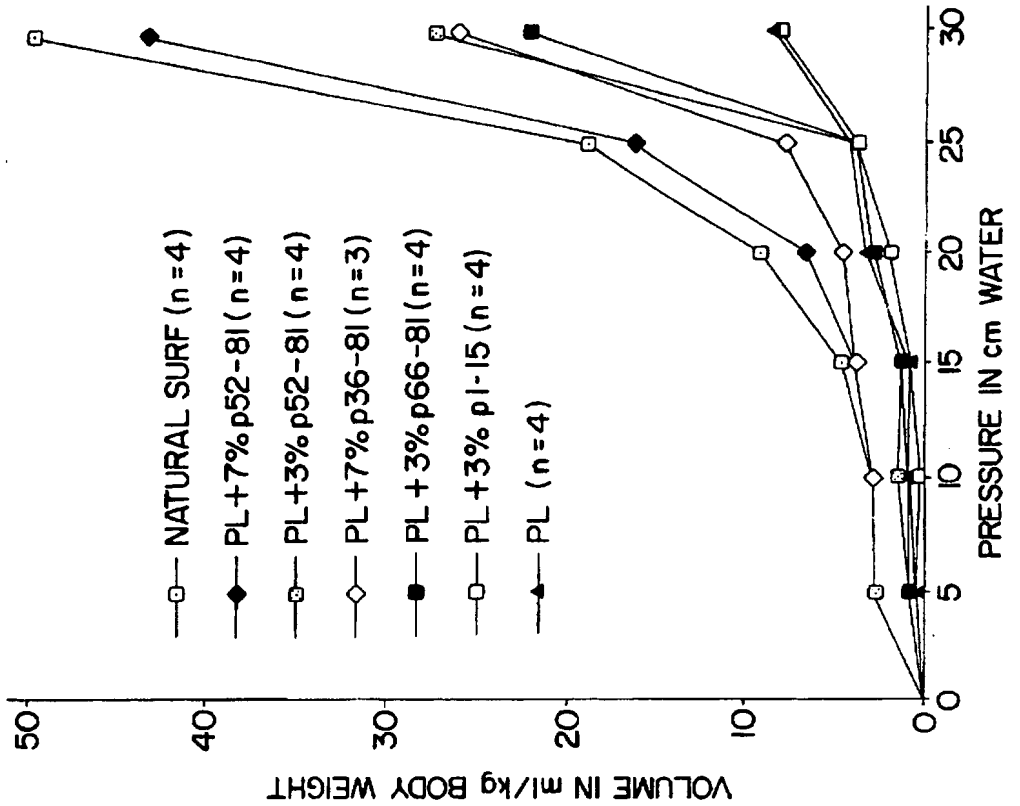
NATURAL SURF (n=4)
PL+7% p52-8l (n=4)
PL+3% p52-8l (n=4)
PL+7% p36-8l (n=3)
PL+3% p66-8l (n=4)
PL+3% pl-15 (n=4)
PL (n=4)
VOLUME IN ml/kg BODY WEIGHT
0
10
20
30
40
50
PRESSURE IN cm WATER
0
5
10
15
20
25
30

FIG. 7A

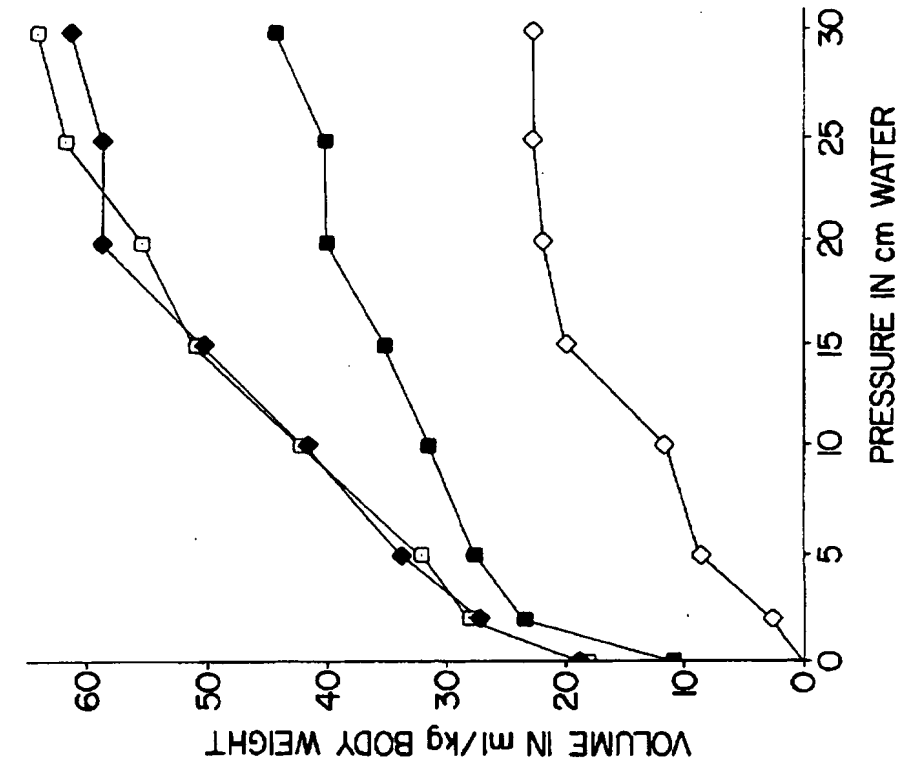
VOLUME IN ml/kg BODY WEIGHT
0
10
20
30
40
50
60
PRESSURE IN cm WATER
0
5
10
15
20
25
30

FIG.8B

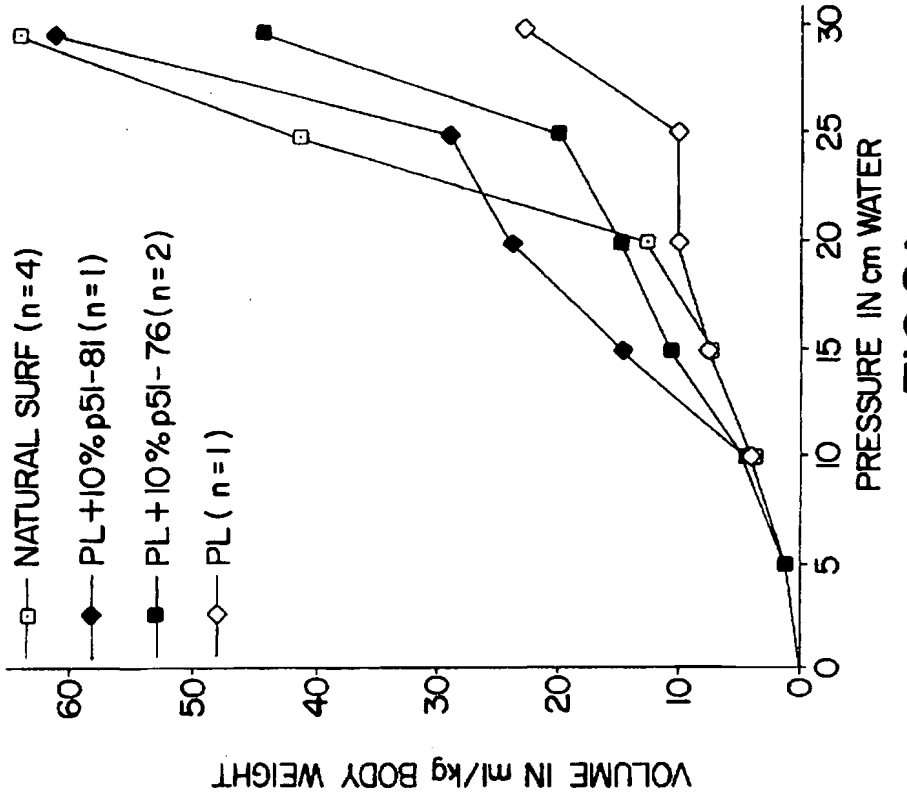
NATURAL SURF (n=4)
PL+10%p5I-8I (n=1)
PL+10%p5I-76 (n=2)
PL (n=1)
VOLUME IN ml/kg BODY WEIGHT
0
10
20
30
40
50
60
PRESSURE IN cm WATER
0
5
10
15
20
25
30

FIG.8A

PULMONARY SURFACTANT PROTEINS AND RELATED POLYPEPTIDES

CROSS REFERENCE TO RELATED APPLICATIONS

This is a continuation of application Ser. No. 08/060,833, filed May 12, 1993 now U.S. Pat. No. 5,407,914, which is a continuation-in-part of application Ser. No. 07/715,397, filed Jun. 14, 1991 (now U.S. Pat. No. 5,260,273), which is a continuation-in-part of application Ser. No. 07/293,201, filed Jan. 4, 1989 (now U.S. Pat. No. 5,164,369), which is a continuation-in-part of application Ser. No. 141,200, filed Jan. 6, 1988 (now abandoned), the disclosures of which are hereby incorporated by reference.

TECHNICAL FIELD

The present invention relates to SP18 monomer-related polypeptides useful in forming synthetic pulmonary surfactants. The present invention also relates to a recombinant nucleic acid molecule carrying a structural gene that encodes human SP18 monomer protein and the use of such a recombinant molecule to produce human SP18 monomer.

BACKGROUND

Pulmonary surfactant (PS) lines the alveolar epithelium of mature mammalian lungs. Natural PS has been described as a "lipoprotein complex" because it contains both phospholipids and apoproteins that interact to reduce surface tension at the lung air-liquid interface.

Since the discovery of pulmonary surfactant, and the subsequent finding that its deficiency is a primary cause of neonatal and adult respiratory distress syndrome (RDS), a number of studies have been directed towards developing effective surfactant replacement therapy for affected individuals, particularly infants, using exogenous PS. For instance, improvements in lung function as measured by a decrease in mean airway pressure and oxygen requirements have been demonstrated using exogenous surfactants in human pre-term infants. See Hallman, et al. *Pediatrics* 71: 473–482 (1983); Merritt, et al. *J. Pediatr.* 108: 741–745 (1986); Hallman, et al. *J. Pediatr.* 106: 963–969 (1985); Morley, et al. *Lancet* 1: 64–68 (1981); Merritt, et al. *New England J. Med.* 315: 785–790 (1986). Smyth, et al. *Pediatrics* 71: 913–917 (1983); Enhorning, et al. *Pediatrics* 76: 145–153 (1985); Fujiwara, et al. *The Lancet* 1: 55–59 (1980); Kwong, et al. *Pediatrics* 76: 585–592 (1985); Shapiro, et al. *Pediatrics* 76: 593–599 (1985); Fujiwara, in "Pulmonary Surfactant", Robertson, B., Van Golde, L.M.G., Batenburg J. (eds), Elsevier Science Publishers, Amsterdam, pp. 479–503, (1984).

From a pharmacologic point of view, the optimal exogenous PS to use in the treatment of RDS would be one completely synthesized in the laboratory, under controlled and sterile conditions, with negligible batch-to-batch variability in properties. To minimize the possibility of immunologic complications, the apoprotein component of an exogenous PS should be identical to that found in humans. Unfortunately, the composition of naturally occurring PS is complex, and the art has not yet identified all of the biochemical components that generate the biophysical properties needed for high physiologic activity in lungs. In particular, the art has failed to characterize all of the apoproteins present in natural PS or identify the function of the PS apoproteins presently known.

It should be noted that the literature on PS apoproteins and their roles in surfactant function is complex, inconsistent and sometimes contradictory because heterogenous apoprotein preparations were used in many studies. To date, the art has not definitively established the number of different apoproteins present in natural PS.

Of particular interest to the present invention is the use of a low molecular weight (LMW) human PS-associated apoprotein as a component in an exogenous surfactant. Several studies have attempted to isolated or define human PS LMW apoproteins using biochemical techniques. See, for example, Phizackerley, et al. *Biochem. J.* 183: 731–736 (1979), Revak, et al. *Am. Rev. Resp. Dis.* 134: 1258–1265 (1986), Suzuki, et al. *Eur. J. Respir. Dis.* 69: 335–345 (1986), Taeusch, et al. *Pediatrics* 77: 572–581 (1986), Yu, et al. *Biochem. J.* 236: 85–89 (1986), Whitsett, et al. *Pediatric Res.* 20: 460–467 (1986), Whitsett, et al. *Pediatric Res.* 20: 744–749 (1986), Takahashi, et al. *Biochem. Biophys. Res. Comm.* 135: 527–532 (1986), Suzuki, et al. *Exp. Lung. Res.* 11: 61–73 (1986), Curstedt, et al. *Eur. J. Biochem.* 168: 255–262 (1987), Notter, et al. *Chem. Phys. Lipids* 44: 1–17 (1987), and Phelps, et al. *Am. Rev. Respir. Dis.* 135: 1112–1117 (1987).

Recently, the art has begun to apply the methods of recombinant DNA technology to overcome the problems associated with not being able to isolate to homogeneity the individual LMW PS apoproteins. For instance, Glasser, et al. *Proc. Nat. Acad. Sci. USA* 84: 4007–4011 (1987) reported a cDNA derived sequence of amino acid residues that forms at least a portion of a human precursor protein from which at least one mature LMW apoprotein, which they designated SPL (Phe), is formed. While Glasser, et al. were not able to determine the carboxy-terminal residue of SPL(Phe), and therefore were not able to identify its complete sequence, they did predict that mature SPL(Phe) was about 60 amino acids in length.

Jacobs, et al. *J. Biol. Chem.* 262: 9808–9811 (1987) have described a cDNA and derived amino acid residue sequence for a human precursor protein similar to that described by Glasser, et al. supra. However, according to Jacobs et al. the mature LMW apoprotein, which they designated PSP-B, formed from the precursor would be 76 amino acid residues in length. In addition, Jacobs, et al. noted that it was not clear that any PS apoprotein derived from the reported precursor protein was present in the surfactant preparations that had been studied clinically by others.

From the foregoing it can be seen that the literature contains multiple nomenclature for what is apparently the same PS apoprotein. Therefore, for ease of discussion, the mature apoprotein derived from the precursor protein described by Glasser, et al. supra, and Jacobs, et al. supra, will be referred to herein generically as "SP18", with the monomeric and dimeric forms being referred to as "SP18 monomer" and "SP18 dimer", respectively, when appropriate.

The canine SP18 precursor has been described by Hawgood, et al. *PNAS USA* 84: 66–70 (1987) and Schilling, et al. International Patent Application WO 86/03408. However, it should be noted that both those studies suffered the same inability to define the mature, biologically active form of SP18 as the Glasser, et al. supra, and Jacobs, et al. supra, studies.

Warr, et al. *PNAS USA* 84: 7915–7919 (1987) describe a cDNA derived sequence of 197 amino acid residues that forms a precursor protein from which a mature LMW apoprotein, they designate as SPS, is formed. Like the studies attempting to describe SP18, Warr, et al. were unable to determine the carboxy terminal residue of the mature

protein formed from the precursor protein sequence, and thus were not able to definitively characterize SP5.

Because the amino acid residue sequence of the precursor protein reported by Warr et al. is different from that reported by Glasser, et al. and Jacobs, et al. it therefore appears that the art has determined that natural PS contains at least two LMW apoproteins. However, the biologically active forms of those proteins has remained undetermined.

SUMMARY

The present invention contemplates a polypeptide comprising at least 10 amino acid residues and no more than about 60 amino acid residues, and preferably is in the range of 20–30 residues in length. The polypeptide includes a sequence having alternating hydrophobic and positively charged amino acid residue regions. The polypeptide is represented by the formula $(Z_aU_b)_cZ_d$.

Z is a positively charged amino acid residue, preferably one R or K; U is a hydrophobic amino acid residue, independently selected from the group consisting of V, I, L, C, Y and F. A preferred hydrophobic residue is L.

The average value of a is about 1 to about 5, and is preferably 1.

The average value of b is about 3 to about 20, and is preferably in the range of 4–8, and more preferably is about 4.

The value of c is 1 to 10, and is preferably in the range of 4–8, and more preferably is about 4.

The value of d is 0 to 3, and is preferably 1.

A preferred polypeptide has an amino acid residue sequence represented by the formula:

KLLLLKLLLLKLLLLKLLLLK (SEQ ID NO 1).

The polypeptide, when admixed with a pharmaceutically acceptable phospholipid, forms a synthetic pulmonary surfactant having a surfactant activity greater than the surfactant activity of the phospholipid alone.

A method of treating respiratory distress syndrome comprising administering a therapeutically effective amount of the synthetic pulmonary surfactant is also contemplated.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 illustrates a 750 nucleotide cDNA sequence (top lines) and deduced amino acid residue sequence (bottom lines). The number to the right of each line-of nucleotides represents the numerical position in the sequence of the nucleotide at the end of each line. The nucleotides are grouped into codons, 15 codons per line, with the amino acid residue coded for by each codon shown in triple letter code directly below the codon. The numerical position of some residues in the amino acid residue sequence encoded by the cDNA is shown below the residues. The amino-terminal amino acid residue of mature human SP18 monomer is Phe (encoded by nucleotides 187–189) and is designated residues number 1. The carboxy-terminal amino acid residue is Asp at residue position 81 (encoded by nucleotides 427–429). A structural gene encoding mature SP18 monomer therefore contains 81 codons and has a nucleotide sequence that corresponds to nucleotides 187–429.

FIG. 2 illustrates the protein elution profile of PS apoproteins from a Bio-Sil HA (silic acid) column. Results of Pierce BCA protein assay (solid line) and phospholipid analyses (broken line) are shown for selected fractions. Two milliliters (ml) were collected per fraction. Positive protein assay in fractions 28 to 33 is due to the presence of phospholipids.

FIG. 3 illustrates a Silver-stained SDS-PAGE of low molecular weight (LMW) PS apoproteins. Lanes A and D show a sample after silicic acid or Sephadex LH-20 chromatography; both LMW proteins are present. Lanes B, C, E and F show the resolution of SP18 (lanes B and E) and SP9 (lanes C and F) following chromatography on Sephadex LH-60. Molecular weight standards are shown in lane G. Lanes A–C are unreduced samples, lanes D–F contain identical samples reduced with β-mercaptoethanol prior to electrophoresis.

FIGS. 4A and 4B, respectively, illustrate inflation and deflation pressure/volume curves of fetal rabbit lungs 30 min after intratracheal instillation of 100 μl of saline (open circles), 2 mg phospholipids (PL) DPPC:PG, 3:1 (closed circles), PL+10 μg SP9 (open squares) PL+10 μg SP18 (closed squares), or 2 mg natural human surfactant (closed triangles). Data are expressed as the mean of 4 animals±one standard deviation.

FIGS. 5A, 5B, 5C, and 5D illustrate fetal rabbit lung tissue samples (×125 magnification, hematoxylin-eosin stain) following treatment with saline (A), natural human surfactant (B), phospholipids DPPC:PG (C) or phospholipids plus LMW apoproteins (SP9+SP18) (D).

FIG. 6 illustrates the surfactant activity of exemplary polypeptide-containing synthetic surfactants of the present invention. Surfactant activity was determined by measuring the pressure gradient across an air/liquid interface using the pulsating bubble technique. The pressure gradient (ΔP) across the surface of the bubble is the absolute value of the pressure recorded in centimeters of H_2O. The results obtained for each synthetic pulmonary surfactant are identified by the polypeptide in the surfactant. Results obtain for surfactants consisting of phospholipid alone (i.e., with no peptide or protein admixed therewith) are identified as PL. The results obtained using a control peptide having only 8 amino acid residues and having a sequence corresponding to human SP18 monomer residues 74–81 (p74–81) are also shown. The data time points shown were obtained at 15 seconds, 1 minute and 5 minutes.

FIGS. 7A and 7B are two graphs that illustrate the results of a static compliance study of exemplary synthetic surfactants of this invention using the fetal rabbit model previously described in Revak, et al. *Am. Rev. Respir. Dis.* 134: 1258–1256 (1986). Following instillation of a synthetic surfactant or control into the trachea, the rabbit was ventilated for 30 minutes prior to making static compliance measurements. The "x" axis represents the pressure in cm of water, while the "y" axis represents the volume in ml/kg of body weight. The graph in FIG. 7A represents values at inflation and the graph on the right (FIG. 7B) represents deflation values. The results for the following tested surfactants are illustrated: natural surfactant (open square with a dot in the center), phospholipid (PL) with 7% p52–81 (a polypeptide corresponding to residues 52 to 81 of SP18) (closed diamonds); PL with 3% P52–81 (closed squares with white dot in center); PL with 7% p36–81 (open diamonds); PL with 3% p66–81 (closed squares); PL with 3% p1–15 (open squares) and PL control (closed triangles).

FIGS. 8A and 8B are two graphs that illustrate the results of a static compliance study of exemplary synthetic surfactants of the invention. The procedure was performed as described in FIG. 7 except that a different instillation procedure was used. The "x" and "y" axis and right and left graphs are as described in FIG. 7. The results for the following tested surfactants are illustrated: natural surfactant (open squares); phospholipid (PL) with 10% p51–81 (closed

diamonds); PL with 10% p51–76 (closed squares); and PL (closed triangles).

DETAILED DESCRIPTION OF THE INVENTION

A. Definitions

Amino Acid: All amino acid residues identified herein are in the natural L-configuration. In keeping with standard polypeptide nomenclature, *J. Biol. Chem.* 243: 3557–59, (1969), abbreviations for amino acid residues are as shown in the following Table of Correspondence:

TABLE OF CORRESPONDENCE

SYMBOL		
1-Letter	3-Letter	AMINO ACID
Y	Tyr	L-tyrosine
G	Gly	glycine
F	Phe	L-phenylalanine
M	Met	L-methionine
A	Ala	L-alanine
S	Ser	L-serine
I	Ile	L-isoleucine
L	Leu	L-leucine
T	Thr	L-threonine
V	Val	L-valine
P	Pro	L-proline
K	Lys	L-lysine
H	His	L-histidine
Q	Gln	L-glutamine
E	Glu	L-glutamic acid
W	Trp	L-tryptophan
R	Arg	L-arginine
D	Asp	L-aspartic acid
N	Asn	L-asparagine
C	Cys	L-cysteine

It should be noted that all amino acid residue sequences are represented herein by formulae whose left to right orientation is in the conventional direction of amino-terminus to carboxy-terminus. Furthermore, it should be noted that a dash at the beginning or end of an amino acid residue sequence indicates a bond to a radical such as H and OH (hydrogen and hydroxyl) at the amino- and carboxy-termini, respectively, or a further sequence of one or more amino acid residues. In addition, it should be noted that a virgule (/) at the right-hand end of a residue sequence indicates that the sequence is continued on the next line.

Polypeptide and Peptide: Polypeptide and peptide are terms used interchangeably herein to designate a linear series of no more than about 60 amino acid residues connected one to the other by peptide bonds between the alpha-amino and carboxy groups of adjacent residues.

Protein: Protein is a term used herein to designate a linear series of greater than about 60 amino acid residues connected one to the other as in a polypeptide.

Nucleotide: a monomeric unit of DNA or RNA consisting of a sugar moiety (pentose), a phosphate, and a nitrogenous heterocyclic base. The base is linked to the sugar moiety via the glycosidic carbon (1' carbon of the pentose) and that combination of base and sugar is a nucleoside. When the nucleoside contains a phosphate group bonded to the 3' or 5' position of the pentose it is referred to as a nucleotide.

Base Pair (bp): A partnership of adenine (A) with thymine (T), or of cytosine (C) with guanine (G) in a double stranded DNA molecule.

B. SP18 Monomer-Containing Compositions

The present invention contemplates a SP18 monomer-containing composition (subject protein composition) wherein the SP18 monomer is present in either substantially isolated or substantially pure form. By "isolated" is meant that SP18 monomer and SP18 dimer are present as part of a composition free of other alveolar surfactant proteins.

By "substantially pure" is meant that SP18 monomer is present as part of a composition free of other alveolar surfactant proteins and wherein less than 20 percent, preferably less than 10 percent and more preferably less than 5 percent, of the SP18 monomer present is in homodimeric form, i.e., present as part of SP18 dimer.

Preferably, a SP18 monomer-containing composition of the present invention contains human SP18 monomer. More preferably, a SP18 monomer-containing composition contains SP18 monomer having an amino acid residue sequence corresponding to the amino acid residue sequence shown in FIG. 1 from about residue position 1 to at least about residue position 75, preferably to at least about position 81. More preferably, a SP18 monomer used to form a subject protein composition corresponds in sequence to the sequence shown in FIG. 1 from residue position 1 to residue position 81.

Preferably, the amino acid residue sequence of a SP18 monomer in a subject SP18 monomer-containing composition corresponds to the sequence of a native SP18 monomer. However, it should be understood that a SP18 monomer used to form a protein composition of the present invention need not be identical to the amino acid residue sequence of a native SP18 monomer, but may be subject to various changes, such as those described hereinbelow for a polypeptide of this invention, so long as such modifications do not destroy surfactant activity. Such modified protein can be produced, as is well known in the art, through, for example, genomic site-directed mutagenesis.

"Surfactant activity" for a protein or polypeptide is defined as the ability, when combined with lipids, either alone or in combination with other proteins, to exhibit activity in the in vivo assay of Robertson, *Lung* 158: 57–68 (1980). In this assay, the sample to be assessed is administered through an endotracheal tube to fetal rabbits or lambs delivered prematurely by Caesarian section. (These "premies" lack their own PS, and are supported on a ventilator.) Measurements of lung compliance, blood gases and ventilator pressure provide indices of activity. Preliminary assessment of activity may also be made by an in vitro assay, for example that of King, et al, *Am. J. Physiol.* 223: 715–726 (1972), or that illustrated below which utilizes a measurement of surface tension at a air-water interface when a protein or polypeptide is admixed with a phospholipid.

C. Nucleic Acid Segments

In living organisms, the amino acid residue sequence of a protein or polypeptide is directly related via the genetic code to the deoxyribonucleic acid (DNA) sequence of the structural gene that codes for the protein. Thus, a structural gene can be defined in terms of the amino acid residue sequence, i.e., protein or polypeptide, for which it codes.

An important and well known feature of the genetic code is its redundancy. That is, for most of the amino acids used to make proteins, more than one coding nucleotide triplet (codon) can code for or designate a particular amino acid residue. Therefore, a number of different nucleotide sequences may code for a particular amino acid residue sequence. Such nucleotide sequences are considered functionally equivalent since they can result in the production of the same amino acid residue sequence in all organisms. Occasionally, a methylated variant of a purine or pyrimidine may be incorporated into a given nucleotide sequence. However, such methylations do not affect the coding relationship in any way.

A DNA segment of the present invention is characterized as consisting essentially of a DNA sequence that encodes a SP18 monomer, preferably human SP18 monomer. That is, a DNA segment of the present invention forms a structural gene capable of expressing a SP18 monomer. While the codons of the DNA segment need not be collinear with the amino acid residue sequence of SP18 monomer because of the presence of an intron, it is preferred that the structural gene be capable of expressing SP18 monomer in mature form, i.e., without the need for post-translational proteolytic processing. Preferably, the gene is present as an uninterrupted linear series of codons where each codon codes for an amino acid residue found in a SP18 monomer, i.e., a gene containing no introns.

Thus, a DNA segment consisting essentially of the sequence shown in FIG. 1 from about nucleotide position 187 to about nucleotide position 426, preferably to about nucleotide position 429, and capable of expressing SP18 monomer, constitutes one preferred embodiment of the present invention.

DNA segments that encode SP18 monomer can easily be synthesized by chemical techniques, for example, the phosphotriester method of Matteucci, et al. *J. Am. Chem. Soc.* 103: 3185 (1981). Of course, by chemically synthesizing the coding sequence, any desired modifications can be made simply by substituting the appropriate bases for those encoding the native amino acid residue sequence.

Also contemplated by the present invention are ribonucleic acid (RNA) equivalents of the above described DNA segments.

D. Recombinant Nucleic Acid Molecules

The recombinant nucleic acid molecules of the present invention can be produced by operatively linking a vector to a nucleic acid segment of the present invention.

As used herein, the phase "operatively linked" means that the subject nucleic acid segment is attached to the vector so that expression of the structural gene formed by the segment is under the control of the vector.

As used herein, the term "vector" refers to a nucleic acid molecule capable of replication in a cell and to which another nucleic acid segment can be operatively linked so as to bring about replication of the attached segment. Vectors capable of directing the expression of a structural gene coding for SP18 monomer are referred to herein as "expression vectors." Thus, a recombinant nucleic acid molecule (rDNA or rRNA) is a hybrid molecule comprising at least two nucleotide sequences not normally found together in nature.

The choice of vector to which a nucleic acid segment of the present invention is operatively linked depends directly, as is well known in the art, on the functional properties desired, e.g., protein expression, and the host cell to be transformed, these being limitations inherent in the art of constructing recombinant nucleic acid molecules. However, a vector contemplated by the present invention is at least capable of directing the replication, and preferably also expression, of SP18 monomer structural gene included in a nucleic acid segment to which it is operatively linked.

In preferred embodiments, a vector contemplated by the present invention includes a procaryotic replicon, i.e., a DNA sequence having the ability to direct autonomous replication and maintenance of an rDNA molecule extrachromosomally in a procaryotic host cell, such as a bacterial host cell, transformed therewith. Such replicons are well known in the art. In addition, those embodiments that include a procaryotic replicon also include a gene whose expression confers drug resistance to a bacterial host transformed therewith. Typical bacterial drug resistance genes are those that confer resistance to ampicillin or tetracycline.

Those vectors that include a procaryotic replicon can also include a procaryotic promoter capable of directing the expression (transcription and translation) of a SP18 monomer gene in a bacterial host cell, such as *E. coli*, transformed therewith. A promoter is an expression control element formed by a DNA sequence that permits binding of RNA polymerase and transcription to occur. Promoter sequences compatible with bacterial hosts are typically provided in plasmid vectors containing convenient restriction sites for insertion of a DNA segment of the present invention. Typical of such vector plasmids are pUC8, pUC9, pBR322 and pBR329 available from Biorad Laboratories, (Richmond, Calif.) and pPL and pKK223 available from Pharmacia, Piscataway, N.J.

Expression vectors compatible with eucaryotic cells, preferably those compatible with vertebrate cells, can also be used to form an rDNA molecule of the present invention. Eucaryotic cell expression vectors are well known in the art and are available from several commercial sources. Typically, such vectors are provided containing convenient restriction sites for insertion of the desired DNA segment. Typical of such vectors are pSVL and pKSV-10 (Pharmacia), pBPV-1/pML2d (International Biotechnologies, Inc.), and pTDT1 (ATCC, #31255).

In preferred embodiments, a eucaryotic cell expression vector used to construct an rDNA molecule of the present invention includes a selection marker that is effective in a eucaryotic cell, preferably a drug resistance selection marker. A preferred drug resistance marker is the gene whose expression results in neomycin resistance, i.e., the neomycin phosphotransferase (neo) gene. Southern, et al. *J. Mol. Appl. Genet.* 1: 327–341 (1982).

The use of retroviral expression vectors to form a recombinant nucleic acid molecule of the present invention is also contemplated. As used herein, the term "retroviral expression vector" refers to a nucleic acid molecule that includes a promoter sequence derived from the long terminal repeat (LTR) region of a retrovirus genome.

In preferred embodiments, the expression vector is a retroviral expression vector that is replication-incompetent in eucaryotic cells. The construction and use of retroviral vectors has been described by Sorge, et al. *Mol. Cell. Biol.* 4: 1730–37 (1984).

A variety of methods have been developed to operatively link nucleic acid segments to vectors via complementary cohesive termini. For instance, complementary homopolymer tracts can be added to the nucleic acid segment to be inserted and to a terminal portion of the vector nucleic acid. The vector and nucleic acid segment are then joined by hydrogen bonding between the complementary homopolymeric tails to form a recombinant nucleic acid molecule.

Synthetic linkers containing one or more restriction sites provide an alternative method of joining a nucleic acid segment to vectors. For instance, a DNA segment of the present invention is treated with bacteriophage T4 DNA polymerase or *E. coli* DNA polymerase I, enzymes that remove protruding, 3', single-stranded termini with their 3'-5' exonucleolytic activities and fill in recessed 3' ends with their polymerizing activities. The combination of these activities therefore generates blunt-ended DNA segments. The blunt-ended segments are then incubated with a large molar excess of linker molecules in the presence of an enzyme that is able to catalyze the ligation of blunt-ended DNA molecules, such as bacteriophage T4 DNA ligase. Thus, the products of the reaction are DNA segments

carrying polymeric linker sequences at their ends. These DNA segments are then cleaved with the appropriate restriction enzyme and ligated to an expression vector that has been cleaved with an enzyme that produces termini compatible with those of the DNA segment.

Synthetic linkers containing a variety of restriction endonuclease sites are commercially available from a number of sources including International Biotechnologies, Inc., New Haven, Conn.

Also contemplated by the present invention are RNA equivalents of the above described recombinant DNA molecules.

E. Transformed Cells and Cultures

The present invention also relates to a host cell transformed with a recombinant nucleic acid molecule of the present invention, preferably an rDNA capable of expressing an SP18 monomer. The host cell can be either procaryotic or eucaryotic.

"Cells" or "transformed host cells" or "host cells" are often used interchangeably as will be clear from the context. These terms include the immediate subject cell, and, of course, the progeny thereof. It is understood that not all progeny are exactly identical to the parental cell, due to chance mutations or differences in environment. However, such altered progeny are included when the above terms are used.

Bacterial cells are preferred procaryotic host cells and typically are a strain of *E. coli* such as, for example, the *E. coli* strain DH5 available from Bethesda Research Laboratories, Inc., Bethesda, Md. Preferred eucaryotic host cells include yeast and mammalian cells, preferably vertebrate cells such as those from a mouse, rat, monkey or human fibroblastic cell line. Preferred eucaryotic host cells include Chinese hamster ovary (CHO) cells available from the ATCC as CCL61 and NIH Swiss mouse embryo cells NIH/3T3 available from the ATCC as CRL 1658. Transformation of appropriate cell hosts with a recombinant nucleic acid molecule of the present invention is accomplished by well known methods that typically depend on the type of vector used. With regard to transformation of procaryotic host cells, see, for example, Cohen, et al. *Proc. Natl. Acad. Sci. USA* 69: 2110 (1972); and Maniatis, et al, *Molecular Cloning, A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. (1982). With regard to transformation of vertebrate cells with recombinant nucleic acid molecules containing retroviral vectors, see, for example, Sorge, et al. *Mol. Cell. Biol.* 4: 1730–37 (1984); Graham, et al. *Virol.* 52: 456 (1973); and Wigler, et al, *PNAS USA* 76: 1373–76 (1979).

Successfully transformed cells, i.e., cells that contain a recombinant nucleic acid molecule of the present invention, can be identified by well known techniques. For example, cells resulting from the introduction of an rDNA of the present invention can be cloned to produce monoclonal colonies. Cells from those colonies can be harvested, lysed and their DNA content examined for the presence of the rDNA using a method such as that described by Southern, *J. Mol. Biol.* 98: 503 (1975) or Berent, et al, Biotech. 3: 208 (1985).

In addition to directly assaying for the presence of rDNA, successful transformation can be confirmed by well known immunological methods when the rDNA is capable of directing the expression of an SP18 monomer. For example, cells successfully transformed with an expression vector operatively linked to a DNA segment of the present invention produce proteins displaying SP18 monomer antigenicity. Thus, a sample of a cell culture suspected of containing transformed cells are harvested and assayed for human SP18 using antibodies specific for that antigen, the production and use of such antibodies being well known in the art.

Thus, in addition to the transformed host cells themselves, the present invention also contemplates a culture of those cells, preferably a monoclonal (clonally homogeneous) culture, or a culture derived from a monoclonal culture, in a nutrient medium. Preferably, the culture also contains a protein displaying SP18 monomer antigenicity, and more preferably, biologically active SP18 monomer.

Nutrient media useful for culturing transformed host cells are well known in the art and can be obtained from several commercial sources. In embodiments wherein the host cell is mammalian, a "serum-free" medium is preferably used.

F. Recombinant Methods for Producing SP18

Another aspect of the present invention pertains to a method for producing SP18, preferably human SP18 monomer. The method entails initiating a culture comprising a nutrient medium containing host cells, preferably human cells, transformed with a rDNA molecule of the present invention that is capable of expressing SP18 monomer. The culture is maintained for a time period sufficient for the transformed cells to express SP18 monomer. The expressed protein is then recovered from the culture.

Methods for recovering an expressed protein from a culture are well known in the art and include fractionation of the protein-containing portion of the culture using well known biochemical techniques. For instance, the methods of gel filtration, gel chromatography, ultrafiltration, electrophoresis, ion exchange, affinity chromatography and the like, such as are known for protein fractionations, can be used to isolate the expressed proteins found in the culture. In addition, immunochemical methods, such as immunoaffinity, immunoadsorption and the like can be performed using well known methods.

Also contemplated by the present invention is an SP18 monomer produced by a recombinant nucleic acid method described herein.

G. Polypeptides

A polypeptide of the present invention (subject polypeptide) is characterized by its amino acid residue sequence and novel functional properties. A subject polypeptide when admixed with a pharmaceutically acceptable phospholipid forms a synthetic pulmonary surfactant having a surfactant activity greater than the surfactant activity of the phospholipid alone (as indicated by a lower ΔP as shown in FIGS. **6**, **7** and **8**).

As seen in FIG. **1**, SP18 has a large hydrophobic region (residues 1 to about 75), followed by a relatively short hydrophilic region at the carboxy terminus (residues 76 to 80, or 81). In referring to amino acid residue numbers of the SP18 sequence, those residues are as illustrated in FIG. **1**.

In one embodiment, a subject polypeptide consists essentially of at least about 10, preferably at least 11 amino acid residues, and no more than about 60, more usually fewer than about 35 and preferably fewer than about 25 amino acid residues that correspond to the sequence of SP18 monomer.

Usually, the amino acid sequence of a polypeptide of this invention will correspond to a single group of contiguous residues in the linear sequence of SP18. However, polypeptides that correspond to more than one portion of the SP18 sequence are also contemplated. Usually at least one sequence that corresponds to at least 10, preferably at least 15, contiguous residues of the hydrophobic region of SP18 will be present in the peptide. A plurality of hydrophobic region amino acid sequences may be present.

A subject polypeptide will preferably include as its carboxy terminal sequence at least 5 contiguous residues in the

linear sequence of SP18 including residue 80. Thus the polypeptides of this invention may include one or more groups of amino acid residues that correspond to portions of SP18 so that a sequence corresponding to a first group of contiguous residues of the SP18 monomer may be adjacent to a sequence corresponding to a second group of contiguous residues from the same or another portion of the SP18 monomer in the polypeptide sequence. Peptides having two or more sequences that correspond to a single group of contiguous amino acid residues from the linear sequence of SP18 is also contemplated.

Exemplary preferred subject polypeptides corresponding in amino acid residue sequence to human SP18 monomer hydrophobic region are shown in Table 1.

TABLE 1

Designation[1]	Amino Acid Residue Sequence
p1–15	FPIPLPYCWLCRALI
p11–25	CRALIKRIQAMIPKG
p21–35	MIPKGALAVAVAQVC
p31–45	VAQVCRVVPLVAGGI
p41–55	VAGGICQCLAERYSV
p46–76	CQCLAERYSVILLDTLLGRMLPQLVCRLVLR
p51–65	ERYSVILLDTLLGRM
p51–72	ERYSVILLDTLLGRMLPQLVCR
p51–76	ERYSVILLDTLLGRMLPQLVCRLVLR
p54–72	SVILLDTLLGRMLPQLVCR
p54–76	SVILLDTLLGRMLPQLVCRLVLR
p61–75	LLGRMLPQLVCRLVL

[1]The designation of each peptide indicates that portion of the amino acid residue sequence of human SP18 monomer, as shown in FIG. 1 to which the peptide sequence corresponds, i.e., it indicates the location of the peptide sequence in the protein sequence.

In preferred embodiments, a subject polypeptide is further characterized as having a carboxy-terminal amino acid residue sequence represented by the formula:

$$—RLVLRCSMDD_Z,$$

wherein Z is an integer having a value of 0 or 1 such that when Z is 0 the D residue to which it is a subscript is absent and when Z is 1 the D residue to which it is a subscript is present. Exemplary preferred "carboxy-terminal polypeptides" are shown in Table 2.

TABLE 2

Designation[1]	Amino Acid Residue Sequence
p71–81	CRLVLRCSMDD
p66–81	LPQLVCRLVLRCSMDD
p59–81	DTLLGRMLPQLVCRLVLRCSMDD
p52–81	RYSVILLDTLLGRMLPQLVCRLVLRCSMDD
P51–81	ERYSVILLDTLLGRMLPQLVCRLVLRCSMDD
P51–80	ERYSVILLDTLLGRMLPQLVCRLVLRCSMD
p36–81	RVVPLVAGGICQCLAERYSVILLDTLLGRMLPQLVCRLVLRCSMDD
p32–81	AQVCRVVPLVAGGICQCLAERYSVILLDTLLGRMLPQLVCRLVLRCSMDD

[1]The designation is the same as in Table 1.

Preferably, a subject polypeptide has an amino acid residue sequence that corresponds to a portion of the sequence shown in FIG. 1. However, it should be understood that a polypeptide of the present invention need not be identical to the amino acid residue sequence of a native SP18 monomer. Therefore, a polypeptide of the present invention can be subject to various changes, such as insertions, deletions and substitutions, either conservative or non-conservative, where such changes provide for certain advantages in their use.

Conservative substitutions are those where one amino acid residue is replaced by another, biologically similar residue. Examples of conservative substitutions include the substitution of one hydrophobic residue such as isoleucine, valine, leucine or methionine for another, or the substitution of one polar residue for another such as between arginine and lysine, between glutamic and aspartic acids or between glutamine and asparagine and the like. The term "conservative substitution" also includes the use of a substituted amino acid in place of an unsubstituted parent amino acid provided that such a polypeptide also displays the requisite binding activity.

In one preferred embodiment, a serine (S) residue is substituted for a cysteine (C) residue, usually at least one of residue positions 71 and 77. Preferably the serine analog has a sequence corresponding to the sequence of residues 51–76 of the SP18 monomer with the substitution at residue 71 or to the sequence of residues 51–81 with serine substitutions at 71 and 77.

When a polypeptide of the present invention has a sequence that is not identical to the sequence of a native SP18 monomer because one or more conservative or non-conservative substitutions have been made, usually no more than about 20 number percent and more usually no more than 10 number percent of the amino acid residues are substituted, except where additional residues have been added at either terminus as for the purpose of providing a "linker" by which the polypeptides of this invention can be conveniently affixed to a label or solid matrix, or carrier. Labels, solid matrices and carriers that can be used with the polypeptides of this invention are described hereinbelow.

Amino acid residue linkers are usually at least one residue and can be 40 or more residues, more often 1 to 10 residues that do not correspond in amino acid residue sequence to a native SP18 monomer. Typical amino acid residues used for linking are tyrosine, cysteine, lysine, glutamic and aspartic acid, or the like. In addition, a polypeptide sequence of this invention can differ from the natural sequence by the sequence being modified by terminal—NH_2 acylation, e.g., acetylation, or thioglycolic acid amidation, terminal-carboxlyamidation, e.g., ammonia, methylamine, etc.

When coupled to a carrier via a linker to form what is known in the art as a carrier-hapten conjugate, a polypeptide of the present invention is capable of inducing antibodies that immunoreact with SP18 monomer. In view of the well established principle of immunologic cross-reactivity, the present invention therefore contemplates antigenically related variants of the polypeptides shown in Tables 1 and 2. An "antigenically related variant" is a polypeptide that includes at least a six amino acid residue sequence portion of a polypeptide from Table 1 or Table 2 and which is capable of inducing antibody molecules that immunoreact with a polypeptide from Table 1 or 2 and an SP18 monomer.

In another embodiment, a polypeptide of this invention has amino acid residue sequence that has a composite hydrophobicity of less than zero, preferably less than or equal to −1, more preferably less than or equal to −2. Determination of the composite hydrophobicity value for a peptide is described in detail in Example 2. These hydrophobic polypeptides perform the function of the hydrophobic region of SP18. In a preferred embodiment, the amino acid sequence mimics the pattern of hydrophobic and hydrophilic residues of SP18.

A preferred hydrophobic polypeptide includes a sequence having alternating hydrophobic and hydrophilic amino acid residue regions and is characterized as having at least 10 amino acid residues represented by the formula: $(Z_aU_b)_cZ_d$.

Z and U are amino acid residues such that at each occurrence, Z and U are independently selected. Z is a hydrophilic amino acid residue, preferably selected from the group consisting of R, D, E and K. U is a hydrophobic amino acid residue, preferably selected from the group consisting of V, I, L, C, Y and F.

"a", "b", "c" and "d" are numbers which indicate the number of hydrophilic or hydrophobic residues. "a" has an average value of about 1 to about 5, preferably about 1 to about 3. "b" has an average value of about 3 to about 20, preferably about 3 to about 12, most preferably about 3 to about 10. "c" is 1 to 10, preferably 2 to 10, most preferably 3 to 6. "d" is 1 to 3, preferably 1 to 2.

By stating that the amino acid residue represented by Z and U is independently selected, it is meant that at each occurrence a residue from the specified group is selected. That is, when "a" is 2, for example, each of the hydrophilic residues represented by Z will be independently selected and thus can include RR, RD, RE, RK, DR, DD, DE, DK, etc. By stating that "a" and "b" have average values, it is meant that although the number of residues within the repeating sequence (Z_aU_b) can vary somewhat within the peptide sequence, the average values of "a" and "b" would be about 1 to about 5 and about 3 to about 20, respectively.

Exemplary preferred polypeptides of the above formula are shown in Table 3A.

TABLE 3A

Designation[1]	SEQ ID NO	Amino Acid Residue Sequence
DL4		DLLLLDLLLLDLLLLDLLLLD
RL4		RLLLLRLLLLRLLLLRLLLLR
RL8		RLLLLLLLLRLLLLLLLLRLL
RL7		RRLLLLLLLRRLLLLLLLRRL
RCL1		RLLLLCLLLRLLLLCLLLR
RCL2		RLLLLCLLLRLLLLCLLLRLL
RCL3		RLLLLCLLLRLLLLCLLLRLLLLCLLLR
KL4	1	KLLLLKLLLLKLLLLKLLLLK
KL8	2	KLLLLLLLLKLLLLLLLLKLL
KL7	3	KKLLLLLLLKKLLLLLLLKKL

[1]The designation is an abbreviation for the indicated amino acid residue sequence.

Also contemplated are composite polypeptides of 10 to 60 amino acid residues. A composite polypeptide consists essentially of an amino terminal sequence and a carboxy terminal sequence. The amino terminal sequence has an amino acid sequence of a hydrophobic region polypeptide or a hydrophobic peptide of this invention, preferably hydrophobic polypeptide, as defined in the above formula. The carboxy terminal sequence has the amino acid residue sequence of a subject carboxy terminal peptide.

A polypeptide of the present invention can be synthesized by any techniques that are known to those skilled in the polypeptide art. An excellent summary of the many techniques available may be found in J. M. Steward and J. D. Young, "Solid Phase Peptide Synthesis", W. H. Freeman Co., San Francisco, 1969, and J. Meienhofer, "Hormonal Proteins and Peptides", Vol. 2, p. 46, Academic Press (New York), 1983 for solid phase peptide synthesis, and E. Schroder and K. Kubke, "The Peptides", Vol. 1, Academic Press (New York), 1965 for classical solution synthesis.

In general, these methods comprise the sequential addition of one or more amino acid residues or suitably protected amino acid residues to a growing peptide chain. Normally, either the amino or carboxyl group of the first amino acid residue is protected by a suitable, selectively removable protecting group. A different, selectively removable protecting group is utilized for amino acids containing a reactive side group such as lysine.

Using a solid phase synthesis as exemplary, the protected or derivatized amino acid is attached to an inert solid support through its unprotected carboxyl or amino group. The protecting group of the amino or carboxyl group is then selectively removed and the next amino acid in the sequence having the complimentary (amino or carboxyl) group suitably protected is admixed and reacted under conditions suitable for forming the amide linkage with the residue already attached to the solid support. The protecting group of the amino or carboxyl group is then removed from this newly added amino acid residue, and the next amino acid (suitably protected) is then added, and so forth. After all the desired amino acids have been linked in the proper sequence, any remaining terminal and side group protecting groups (and solid support) are removed sequentially or concurrently, to afford the final polypeptide.

H. Synthetic Surfactants

Recombinantly produced SP18 and/or a subject polypeptide can be admixed with a pharmaceutically acceptable phospholipid to form a synthetic pulmonary surfactant (PS) useful in the treatment of respiratory distress syndrome.

The phase "pharmaceutically acceptable" refers to molecular entities and compositions that do not produce an allergic or similar untoward reaction when administered to a human.

Phospholipids useful in forming synthetic alveolar surfactants by admixture with protein are well known in the art. See, Notter, et al, *Clin. Perinatology* 14: 433–79 (1987), for a review of the use of both native and synthetic phospholipids for synthetic surfactants.

In one embodiment, the present invention contemplates a synthetic pulmonary surfactant effective in treating RDS comprising an effective amount of a subject polypeptide admixed with a pharmaceutically acceptable phospholipid. While methods for determining the optimal polypeptide:phospholipid weight ratios for a given polypeptide-phospholipid combination are well known, therapeutically effective ratios are in the range of about 1:5 to about 1:10,000, preferably about 1:100 to about 1:5,000, and more preferably about 1:500 to about 1:1000. In a more preferred embodiment, the polypeptide:phospholipid weight ratio is in the range of about 1:5 to about 1:2,000, preferably about 1:7 to about 1:1,000, and more preferably about 1:10 to about 1:100. Thus, a synthetic pulmonary surfactant of this invention can contain about 50, usually about 80, to almost 100 weight percent lipid and about 50, usually about 20, to less than 1 weight percent polypeptide. Preferably a subject polypeptide is about 1 to about 10 weight percent of the surfactant for polypeptides corresponding to portions of the SP18 sequence and 1:100 for polypeptides corresponding to the entire SP18 monomer.

The lipid portion is preferably about 50 to about 90, more preferably about 50 to about 75, weight percent dipalmi-

toylphosphatidylcholine (DPPC) with the remainder unsaturated phosphatidyl choline, phosphatidyl glycerol (PG), triacylglycerols, palmitic acid sphingomyelin or admixtures thereof. The synthetic pulmonary surfactant is prepared by admixing a solution of a subject polypeptide with a suspension of liposomes or by admixing the subject polypeptide and lipids directly in the presence of organic solvent. The solvent is then removed by dialysis or evaporation under nitrogen and/or exposure to vacuum.

A subject synthetic pulmonary surfactant is preferably formulated for endotracheal administration, e.g., typically as a liquid suspension, as a dry powder "dust", or as an aerosol. For instance, a synthetic surfactant (polypeptide-lipid micelle) is suspended in a liquid with a pharmaceutically acceptable excipient such as water, saline, dextrose, glycerol and the like. A surfactant-containing therapeutic composition can also contain small amounts of non-toxic auxiliary substances such as pH buffering agents including sodium acetate, sodium phosphate and the like. To prepare a synthetic surfactant in dust form, a synthetic surfactant is prepared as described herein, then lyophilized and recovered as a dry powder.

If it is to be used in aerosol administration, a subject synthetic surfactant is supplied in finely divided form along with an additional surfactant and propellent. Typical surfactants which may be administered are fatty acids and esters. However, it is preferred, in the present case, to utilize the other components of the surfactant complex DPPC and PG. Useful propellants are typically gases at ambient conditions, and are condensed under pressure. Lower alkane and fluorinated alkane, such as Freon, may be used. The aerosol is packaged in a container equipped with a suitable valve so that the ingredients may be maintained under pressure until released.

A synthetic surfactant is administered, as appropriate to the dosage form, by endotracheal tube, by aerosol administration, or by nebulization of the suspension or dust into the inspired gas. Amounts of synthetic PS between about 1.0 and about 400 mg/kg, preferably about 50 mg to about 500 mg/kg, are administered in one dose. For use in newly born infants, one or two administrations are generally sufficient. For adults, sufficient reconstituted complex is administered to produce a PO_2 within the normal range (Hallman, et al. *J. Clinical Investigation* 70: 673–682, 1982).

The following examples are intended to illustrate, but not limit, the present invention.

EXAMPLES

Example 1

Isolation and Characterization of Native SP18

A. Methods

1. Purification of LMW apoproteins

Human pulmonary surfactant was isolated from full-term amniotic fluid and applied to a column of DEAE-Sephacel A-50 (Pharmacia, Uppsala, Sweden) using 4 milliliter (ml) packed volume per 200 milligram (mg) surfactant, in a tris-EDTA buffer containing 1% n-octyl-beta-D-glucopyranoside as described by Revak, et al, *Am. Rev. Respir. Dis.* 134: 1258–1265 (1986) and Hallman, et al, *Pediatrics* 71: 473–482 (1983). This particular column and conditions were used in order to isolate the 35,000 dalton apoprotein (for use in other studies) without exposing it to potentially denaturing organic solvents. The void volume, containing the lipids and proteins which did not bind to the column under these conditions, was pooled and extracted with an equal volume of 2:1 chloroform:methanol.

Following centrifugation to separate the phases, the upper phase (water+methanol) was re-extracted with ½ volume chloroform. After centrifugation, the resultant lower organic phase was added to the initial lower phase and evaporated to dryness under a stream of nitrogen. This extract, which contained 100–180 mg phospholipid, LMW apoproteins, and octylglucopyranoside, was redissolved in 2.5 ml of chloroform:methanol, 2:1.

Following the method of Takahashi, et al, *Biochem. Biophys. Res. Comm.* 135: 527–532 (1986), which was found to afford a good separation of octylglucopyranoside from the LMW proteins and phospholipids, a glass column 2.5 cm in diameter was packed at 4° to a height of 38 cm with Sephadex LH-20 (Pharmacia, Uppsala, Sweden) in 2:1 chloroform: methanol. The sample was loaded and 2 ml fractions collected as chloroform:methanol, 2:1, was run through at a flow rate of 8.5 ml/hr. Phospholipids eluted after 40 ml of buffer had passed through the column. Octylglucopyranoside appeared at the 56–116 ml region.

The phospholipid region was pooled, dried under nitrogen, and redissolved in 1 ml chloroform. A silicic acid column was prepared by packing 9 ml of Bio-Sil HA (BioRad, Richmond, Calif.) in chloroform in a glass column at room temperature. The sample (which contained approximately 50 mg phospholipid) was applied and washed with 11 ml chloroform. A linear gradient of increasing methanol was established using an equal weight of chloroform and methanol (38.8 g, 26.5 ml chloroform and 50 ml methanol). Fractions of 2 ml were collected as the gradient was applied to the column. FIG. 1 shows the protein and phospholipid profiles obtained.

Phospholipid analyses showed a small peak in fractions 17–20 and a major peak after fraction 30. The Pierce BCA protein assay was positive in fractions 12–19 and 28–33, but it should be noted that the latter peak is likely to be due to the phospholipid present in this region. Electrophoresis in sodium dodecyl sulfate polyacrylamide gels showed the LMW apoproteins were present in fractions 13–19 with some separation occurring between SP9 and SP18.

Alternatively, a method devised by Hawgood, et al, *Proc. Natl. Acad. Sci.* 85: 66–70 (1987) employing a butanol extraction of PS followed by chromatography of Sephadex LH-20 in an acidified chloroform/methanol buffer, can be used to isolate the LMW apoprotein mixture. For some studies, a separation of the two LMW apoproteins was effected using Sephadex LH-60. A glass column of 1 cm diameter was packed to 40 cm with Sephadex LH-60 (Pharmacia, Uppsala, Sweden) in chloroform/methanol, 1:1, containing 5% 0.1N HCl. A flow rate of 1–2 ml/hr was used. A mixture of the LMW apoproteins containing about 200–700 micrograms (μg) of protein from either the Bio-Sil HA column or the LH-20 column described by Hawgood, et al, *Proc. Natl. Acad. Sci.* 85: 66–70 (1987), in a volume of 0.5 ml buffer, was applied to the top of the column and fractions of 0.5 ml were collected. Typically, SP18 protein eluted in fractions 16–19 and SP9 in fractions 24–29. Appropriate fractions were pooled and dried in glass tubes under nitrogen. A brief period of lyophilization ensured complete removal of the HCl. Proteins were re-solubilized in methanol prior to use.

2. SDS-Gel Electrophoresis

Gel electrophoresis in 16% polyacrylamide was performed in the presence of sodium dodecyl sulfate (SDS-PAGE) according to the method of Laemmli, *Nature* 227: 680–685 (1970), using 3×7 cm minislab gels. 1% β-mercaptoethanol was added to samples where indicated as a disulfide reducing agent. Following electrophoresis, the

gels were fixed overnight in 50% methanol +12% acetic acid, washed in water for 2 hours, and silver-stained according to the method of Wray, et al. *Anal. Biochem.* 118: 197–203 (1981).

3. Octylglucopyranoside Assay

An assay for the quantitation of n-octyl-beta-D-glucopyranoside, based on the anthrone method of Spiro, Methods Enzymol. 8: 3–5 (1966) has been described previously by Revak, et al. *Am. Rev. Respir. Dis.* 134: 1258–1265 (1986).

4. Protein Determinations

Organic samples containing up to 5 μg protein were dried in 12×75 mm glass tubes under nitrogen. Fifteen microliters (μl) of 1% SDS in H_2O and 300 μl BCA Protein Assay Reagent (Pierce Chemical Co., Rockford, Ill.) were admixed with the protein in each tube. Tubes were covered and incubated at 60° C. for 30 min. After cooling, the samples were transferred to a 96-well flat-bottom polystyrene microtiter plate and OD_{550} measured. Bovine serum albumin was used as a standard. It should be noted that some phospholipids will react in the BCA protein assay making protein quantitations inaccurate when lipid is present (i.e., prior to Bio-Sil HA chromatography). Additionally, once purified, the hydrophobic LMW apoproteins themselves react poorly with the BCA reagents and all quantitations of the isolated proteins were, therefore, based on amino acid compositions.

5. Phospholipids

Dipalmitoylphosphatidylcholine (DPPC, beta, gamma-dipalmitoyl-L-alpha-lecithin) and L-alpha-phosphatidyl-DL-glycerol (PG, derivative of egg lecithin) were purchased from either Calbiochem-Behring (La Jolla, Calif.) or Avanti Polar-Lipids, Inc. (Birmingham, Ala.). DPPC was added to PG in chloroform in a weight ratio of 3:1.

6. Admixture of LMW Apoproteins with Phospholipids

For in vitro assays, a methanol solution containing 4 μg of SP9 or SP18, was added to 400 μg DPPC:PG in chloroform in a 12×75 mm glass tube. Following a brief vortex mixing, the samples were dried under N_2. Ninety microliters of water were added to each and the tubes placed in a 37° C. water bath for 15 minutes, with periodic gentle mixing. Isotonicity was restored with the addition of 10 μl of 9% NaCl to each sample prior to assay. For in vivo rabbit studies, 50 μg LMW apoproteins (containing both SP9 and SP18) or 25 μg SP9 or 25 μg SP18 were dried under N_2. Five mg of phospholipids (DPPC:PG, 3:1) were added in chloroform. The samples were mixed, dried, and resuspended in 250 μl 100 millimolar (mM) saline containing 1.5 mM $CaCl_2$, to yield a reconstituted surfactant at 20 mg/ml with 0.5–1% protein.

7. Surfactant Activity Assays

In vitro assays of surfactant activity, assessed as its ability to lower the surface tension of a pulsating bubble, and in vivo assays utilizing fetal rabbits, have both been described in detail previously by Revak, et al. *Am. Rev. Respir. Dis.* 134: 1258–1265 (1986).

8. Morphometric Analyses

Fetal rabbit lungs, inflated to 30 cm H_2O and then deflated to 10 cm H_2O, were submerged in 10% formalin for 72 hours. Paraffin sections were oriented from apex to base and 5 micron sections taken anterior to posterior. After hematoxylin and eosin staining, 10 fields (100 x) were point-counted from apex to base on multiple sections. Standardized morphometric methods (Weiber, in "Stereological Methods," Vol. I, Academic Press, New York, pp. 33–58, 1979) were used to determine ratios of lung interstitium to air spaces for each treatment group. Intersections of alveolar perimeters were also determined.

9. Phospholipid Phosphorus Assays

Phospholipids were quantitated according to the method of Bartlett, *J. Biol. Chem.* 234: 466–468 (1959).

10. Amino Acid Analysis

Triplicate samples for amino acid compositions were hydrolyzed with HCl at 110° C. for 24 hours, with HCl at 150° C. for 24 hours, or in performic acid at 110° C. for 24 hours followed by HCl hydrolysis at 110° C. for 24 hours. Analyses were performed on a Beckman model 121-M amino acid analyzer (Beckman Instruments, Fullerton, Calif.). Tryptophan was not determined.

11. Amino Acid Sequencing

Vapor-phase protein sequencing was performed on an Applied Biosystems 470A Amino Acid Sequencer (Applied Biosystems, Inc., Foster City, Calif.) with an on-line Model 120A HPLC.

12. Isolation of cDNA Clones for Human SP18

RNA was prepared according to Chirgwin, et al. *Biochemistry* 18: 5294–5299 (1979) from a sample of unaffected adult lung tissue obtained during surgical removal of a neoplastic lesion. Preparation of double stranded cDNA was carried out using standard techniques (Chirgwin et al., supra, and Efstratiadis et al., in "Genetic Engineering", eds. Stelow and Hollaender, Plenum, N.Y., 1: 15–49 (1979) and a library was constructed in lambda NM607 as described by Le Bouc, et al. *F.E.B.S. Letts.* 196: 108–112 (1986). SP18 clones were identified by screening phage plaques with synthetic oligonucleotide probes (Benton, et al. *Science* 196: 180–182 (1977) which were prepared using an Applied Biosystems automated synthesizer and purified by HPLC. Initial candidate clones were obtained using probe TG996 (5'CATTGCCTGTGGTATGGCCTGCCTCC 3') which was derived from the partial nucleotide sequence of a small human surfactant apoprotein cDNA (Schilling et al., International Patent Application WO 86/03408). Larger clones (up to 1.5 kb) were isolated using probe TG1103 (5'TCGAGCAGGATGACGGAGTAGCGCC 3') which was based on the 5' sequence of one of the original clones. The nucleotide sequence of the cDNA clones was determined by the chain termination method (Sanger, et al. *PNAS USA* 74: 5463–5467 (1977) using EcoRI restriction fragments subcloned in an appropriate M13 vector.

B. Results

1. Characteristics of the LMW Apoproteins

The LMW apoproteins isolated from human amniotic fluid appeared after silicic acid chromatography, or after the Sephadex LH-20 column chromatography FIG. 2 described by Hawgood, et al. *PNAS* 85: 66–70 (1987), as two protein bands in SDS-polyacrylamide gel electrophoresis under non-reducing conditions. The upper band, having an apparent molecular weight of 18,000 daltons is a dimer, and therefore designated SP18 dimer. With the addition of β-mercaptoethanol, SP18 dimer reduced to 9,000 daltons and was designated SP18 monomer (FIG. 3). The other LMW apoprotein, designated SP9, appears as a diffuse band between 9 and 12,000 daltons in the presence or absence of reducing agents. SP9 was separated from SP18 dimer and SP18 monomer by chromatography on Sephadex LH-60. The resultant purified proteins are shown in FIG. 3.

Amino acid compositions were determined for SP18 monomer and SP9. Because of the extremely hydrophobic nature of these proteins, HCl hydrolysis was performed at 150° C. for 24 hours, in addition to the standard 110° C. for 24 hour hydrolysis, and values for valine, leucine, and isoleucine calculated from analyses of the hydrolysates done

under the extreme conditions. As shown in Table 3B, both proteins are extremely hydrophobic with high levels of valine and leucine.

TABLE 3B

Amino Acid Composition of Human SP9 and SP18 monomer and a Comparison with the Theoretical Composition of SP18[1]

Amino Acid	SP9 (mole %)	SP18 (mole %)	SP18[1] (mole %)
Aspartic acid (or Asparagine)	1.1	3.4	3.7
Threonine	0.8	1.5	1.2
Serine	1.8	2.7	2.5
Glutamic acid (or Glutamine)	1.5	6.7	6.2
Proline	8.3	7.8	7.4
Glycine	10.6	6.1	4.9
Alanine	4.9	10.2	9.9
Cysteine[2]	9.1	7.2	8.6
Valine[3]	12.2	11.7	11.1
Methionine	3.4	3.2	3.7
Isoleucine[3]	6.8	6.4	7.5
Leucine[3]	22.4	17.4	17.3
Tyrosine	0.7	2.2	2.5
Phenylalanine	2.6	1.5	1.2
Histidine	5.4	0.0	0.0
Lysine	4.7	3.0	2.5
Arginine	3.9	9.0	8.6
Tryptophan	N.D.[4]	N.D.[4]	1.2

[1]The theoretical composition is based on sequence data through residue 81.
[2]Cysteine content was determined following performic acid and HCl hydrolyses.
[3]Isoleucine and leucine content were each determined following 24 hour HCl hydrolysis at 150° C.
[4]Tryptophan was not determined.

Amino-terminal sequence analysis of SP18 monomer yielded the following sequence:

NH_2-Phe-Pro-Ile-Pro-Leu-Pro-Try-.

Repeated sequencing of the purified SP9 monomer showed multiple peptides, all rich in leucine and containing at least six consecutive valines. NH_2-terminal analysis showed phenylalanine, glycine, and isoleucine with the relative amounts of each varying from preparation to preparation.

2. Nucleotide Sequence Analysis of SP18 cDNA

The nucleotide sequence of a SP18 monomer cDNA clone is presented in FIG. 1. The sequence displays 83% homology with the canine SP18 cDNA (Hawgood, et al, *PNAS USA* 85: 66–70 (1987). A sequence within a large open reading frame was identified which matches perfectly with the amino terminus of SP18 monomer as determined by Edman degradation of the isolated protein (underlined in FIG. 3). This suggests that mature SP18 monomer arises by processing of a larger precursor molecule. In the mature sequence there is a single potential N-glycosylation site (Asn 110), no sites for tyrosine sulfation, and no G-X-Y repeats as found in the 35.000 dalton apoprotein (White, et al, *A. Ped. Res.* 19: 501–508 (1985)).

The molecular weight of 9000 daltons obtained by SDS-PAGE of reduced SP18 dimer is lower than that predicted for the complete precursor protein sequence with amino terminus NH2-Phe-Pro-Ile-Pro-Leu-Pro-Tyr (19,772 daltons), implying further processing in the region of amino acids 70–90. In support of this, the theoretical amino acid composition (Table I) of a putative 9000 dalton protein comprising residues 1 to 81 compares well with the determined values for purified SP18 monomer. The amino terminal portion of the precursor protein (residues 1 to 81) is alkaline and more hydrophobic than the COOH terminal portion (residues 82 to 181): the Kyte-Doolittle index for residues 1 to 81 is 9100 (pI 8.6) and is −3000 (pI 5.91) for residues 82 to 181 (Kyte, et al, *J. Pediatrics* 100: 619–622 (1982)). The amino terminus (residues 1 to 81) is, as in the canine sequence (Hawgood, et al, *PNAS* 85: 66–70 (1987)), composed of three hydrophobic domains: residues 1 to 11, 22 to 49 and 53 to 74. These are interspersed with a charged domain (residues 12 to 21) and two hydrophilic and charged stretches (residues 47 to 54 and 72 to 81).

3. Reconstitution of Surfactant Activity with LMW Apoproteins

Samples were prepared containing 400 μg/100 μl phospholipids (DPPC:PG, 3:1 by weight), phospholipids plus 4 μg SP9, or phospholipids plus 4 μg SP18. Each sample was assayed in the pulsating bubble surfactometer for the ability to lower surface tension. The results are shown in Table 4 as the mean minimal surface tension at 15 sec, 1 minute (min), and 5 min. Natural human surfactant, isolated from term amniotic fluid, diluted to 4 mg/ml is shown for comparison. While neither phospholipids nor LMW apoproteins alone had significant surface-tension lowering capacities, a mixture of phospholipids with either SP9 or SP18 showed significant activity. Recombining the phospholipids with 1% by weight of SP18 lowered the surface tensions measured to levels comparable to those obtained with an equal amount of natural human surfactant (6.3±0.2 dynes/cm for phospholipids+SP18 at 15 sec, 2.0±1.2 dynes/cm for natural surfactant). On an equal weight basis, SP9 lowered surface tension less effectively (16.7±0.8 dynes/cm at 15 sec).

TABLE 4

Minimum Surface Tensions in the Pulsating Bubble[1]

	15 sec	1 min	5 min
PL[2]	42.9 ± 1.4	41.6 ± 1.6	34.9 ± 4.9
PL + SP9[3]	16.7 ± 0.8	14.1 ± 1.2	12.2 ± 1.0
PL + SP18[3]	6.3 ± 0.2	5.1 ± 1.0	4.9 ± 0.6
natural human surfactant[4]	2.0 ± 1.2	2.4 ± 1.4	0.4 ± 0.4

[1]Pulsation of 20 cycles/min started 10 sec after bubble formation. All values are in dynes · cm^{-1} and are the average of at least 3 determinations.
[2]Phospholipids DPPC:PG, 3:1, 4 mg/ml
[3]1% by weight compared to phospholipids
[4]diluted to 4 mg/ml

In vivo assays of exogenous (synthetic) surfactant activity were performed by instilling into the airways of immature fetal rabbits saline solutions containing Ca^{++} alone or with the addition of phospholipids, phospholipids plus LMW apoproteins, or natural human surfactant. The animals were ventilated for 30 min and then degassed by placement in a bell jar under vacuum. The lungs were then inflated to given pressures and the volume of air required for each pressure was noted. The volumes required for given pressures during deflation from 30 cm H_2O were likewise determined. The resulting pressure/volume curves are shown in FIGS. 4A and 4B for animals which received synthetic surfactant made with purified SP9 or SP18 (0.5% by weight compared with total phospholipid concentration) and appropriate control animals. Improved lung compliance is apparent in those animals treated with natural or either synthetic surfactant as compared with those receiving saline or phospholipids with the SP18 appearing more effective than SP9 on an equal weight basis. A similar study was performed using a mixture of SP9 and SP18. The results were almost identical to the phospholipid plus SP18 curve presented in FIGS. 4A and 4B.

Following compliance measurements, the lungs were inflated to 30 cm H_2O, deflated back to 10 cm H_2O,

clamped, excised and fixed in formalin. Thin sections were stained with hematoxylin and eosin and examined microscopically. As shown in FIGS. 5A, 5B, 5C, and 5D, lungs treated with saline (A) or phospholipids (C) appeared atelectatic while those from animals which received natural (B) or reconstituted (D) surfactant showed normal alveolar expansion. Morphometric analyses of the thin sections showed an interstitium to air space ratio of 4.70 for saline treatment and 3.29 for phospholipids alone as compared with 0.498 for natural surfactant and 0.538 for reconstituted surfactant. These data are shown in Table 5 and corroborate the significant ($p<0.001$; Mann-Whitney U Test) increase in air space seen in FIGS. 5A, 5B, 5C, and 5D. A comparison of alveolar perimeters similarly demonstrated a significantly ($p<0.003$) greater number of intersections of the alveolar boundaries in saline- or phospholipid-treated fetuses compared to surfactant-treated animals.

TABLE 5

Morphometric Analysis of Airspace Following Fetal Rabbit Treatment

Tracheal Instillation	Interstitium/Air Space
saline	4.70
phospholipids[1]	3.29
phospholipids[1] + LMW Apoproteins[2]	0.538
natural human surfactant[3]	0.498

[1]2 mg of 3:1 DPPC:PG per animal
[2]20 μg of LMW apoproteins added to phospholipids
[3]2 mg per animal

C. Discussion

This study describes two low molecular weight apoproteins isolated from human amniotic fluid surfactant which can be added to known phospholipids to produce a biologically active pulmonary surfactant. While the proteins in the current study have been designated as SP18 dimer, SP18 monomer and SP9, it is apparent from the recent literature that multiple nomenclature and an assortment of reported molecular weights for LMW PS apoproteins (ranging from 5–18,000 daltons) exist. The apparent differences in physical properties may be explained by a variety of factors including species differences, varying purification and handling techniques, varying determinations of low molecular weights based on standards in SDS-polyacrylamide gels, and potential interference by lipids of low molecular weight protein bands in gels. Comparisons of amino acid compositions and sequences and immunologic analyses using monospecific antibodies will help to sort out the LMW apoproteins.

It is felt that the SP9 protein described herein, giving a diffuse band on SDS-polyacrylamide gels from 9–12,000 daltons under reducing or non-reducing conditions, is probably the same protein as that designated SAP-6 by Whitsett, et al, *Pediatric Research* 20: 744–749 (1986), SP5–8 by Hawgood, et al, *PNAS USA* 85: 66–70 (1987), PSP-6 by Phelps, et al, *Am. Rev. Respir. Dis.* 135: 1112–1117 (1987), and the 5 kDa proteolipid of Takahashi, et al, *Biochem. Biophys Res. Comm.* 135: 527–532 (1986). The extremely hydrophobic nature of this protein is apparent from its amino acid composition (Table 3B) and sequence data, showing at least six consecutive valine residues preceded by a leucine-rich region. The presence of three amino-terminal residues (phenylalanine, glycine, and isoleucine) in the preparations of SP9 derived herein from amniotic fluid surfactant suggests a collection of peptides having an identical sequence but having had one or two residues removed from the amino-terminus. Phelps, et al. *Am. Rev. Respir. Dis.* 135: 1112–1117 (1987) have recently reported a similar finding with bovine PSP-6 apoprotein.

SP18 dimer is comprised of two identical 9000 dalton peptides (but different from the 9000 dalton peptide of SP9) that are disulfide linked. The amino acid composition of SP18 monomer (Table 3B) shows a high number of hydrophobic residues. When unreduced SDS-PAGE were overloaded with SP18 protein, sequentially less intensely staining bands were seen at 36,000 and 56,000 daltons, suggesting oligomeric forms of the protein; upon reduction, only a single 9000 dalton band was seen.

Both SP9 and SP18 dimer apoproteins isolated as described above, could be shown to have biophysical activity following recombination with phospholipids. The addition of 1% by weight of SP18 dimer to the phospholipids DPPC:PG resulted in an immediate increase in surface pressure resulting in surface tensions of less than 10 dynes/cm by 15 sec. The addition of 1% SP9 to DPPC:PG was slightly less effective, lowering surface tensions to 16.7, 14.1, and 12.2 dynes/cm at 15 sec, 1 and 5 min, respectively. Mixtures of both SP18 dimer and SP9 were also effective but further studies will be required to determine whether the combined effect is additive or synergistic.

In vivo studies of reconstituted surfactant using the fetal rabbit model (Schneider, et al, *J. Pediatrics* 100: 619–622; 1982) were performed using mixtures of SP18 dimer and SP9 as well as each protein individually. A marked improvement in lung compliance was seen in animals treated with natural surfactant or reconstituted surfactant prepared with SP18 dimer apoprotein, as compared with those receiving phospholipids alone or saline (FIGS. 4A and 4B). A moderate improvement was seen when SP9 was used. Identical studies using a mixture of SP18 dimer and SP9 to prepare the reconstituted surfactant showed results very similar to those obtained with SP18 dimer alone (solid squares, FIGS. 4A and 4B); however, the exact ratio of SP18 dimer and SP9 in those studies could not be accurately ascertained. FIGS. 5A, 5B, 5C, and 5D show representative microscopic alveolar fields indicating the lack of atelectasis following surfactant instillation.

Suzuki, et al. (*Eur. J. Respir. Dis.* 69: 336–345; 1986) have reported a reduction in surface tension (measured on the Wilhelmy balance or in a pulsating bubble) and a five fold increase in tidal volumes of prematurely-delivered rabbits at insufflation pressures of 25 cm and H_2O when porcine LMW (<15,000 daltons) surfactant apoproteins are added to mixtures of DPPC:PG) at a weight ratio of 5:80:20 (protein:DPPC:PG). Whether one or multiple proteins are present in this system is unclear.

Previous studies using the 35,000 dalton apoprotein (Revak, et al, *Am. Rev. Respir. Dis.* 134: 1258–1265; 1986) also showed moderate reduction in surface tension, similar to that obtained with SP9 in the studies described herein. Clearly, further studies must be done using various combinations and concentrations of SP18, SP9 and the 35,000 dalton apoprotein, as well as Ca^{++} and perhaps various phospholipids to elucidate the interactions between these various components of surfactant and to determine the best conditions for a biologically active exogenous surfactant.

Example 2

In Vitro Assessment of Polypeptide Surfactant Activity

A. Methods

1. Measurement of Surfactant Activity

Measurements of surface pressure across an air-liquid interface (expressed in negative cm of H_2O pressure) at minimal (γmin) bubble radius were determined at various times using the pulsating bubble technique described by Enhorning, *J. Appl. Physiol.* 43: 198–203 (1977).

Briefly, the Enhorning Surfactometer (Surfactometer International, Toronto, Ontario) measures the pressure gra-

dient (ΔP) across a liquid-air interface of a bubble that pulsates at a rate of 20 cycles/min between a maximal (0.55 mm) and minimal (0.4 mm) radius. The bubble, formed in a 37° C., water-enclosed, 20-μl sample chamber, is monitored through a microscopic optic while the pressure changes are recorded on a strip chart recorder calibrated for 0 and −2 cm H_2O.

2. Determination of Composite Hydrophobicity Value

The composite hydrophobicity value of each peptide was determined by assigning to each amino acid residue in a peptide its corresponding hydrophilicity value as described in Table 1 of Hopp, et al. *PNAS USA* 78: 3824–3829 (1981), which disclosure is incorporated herein by reference. For a given peptide, the hydrophilicity values were summed, the sum representing the composite hydrophobicity value.

3. Preparation of Synthetic Surfactants

After admixture with solvent, each peptide was combined with phospholipids (DPPC:PG), 3:1 to form a synthetic surfactant according to one of the following methods.

Method A was accomplished by admixing 16 μl of peptide/solvent admixture (40 μg peptide) with 100 μl of chloroform containing 400 μg phospholipids, agitating the admixture for about 10 at 37° C. to form a peptide/phospholipid admixture. Chloroform was removed from the peptide/phospholipid admixture by drying under N_2. The synthetic surfactant thus formed was then admixed with 90 μl of H_2O and gently agitated for about 10 minutes at 37° C. Subsequently, 10 μl of 9% NaCl was admixed to the surfactant-containing solution.

Method B was accomplished by first placing 100 μl of chloroform containing 400 μg of phospholipids in a glass tube and removing the chloroform by drying under N_2 for about 10 minutes at 37° C. Sixteen μl of peptide/solvent admixture and 74 μl H_2O were admixed with the dried phospholipids, and then gently agitated for about 10 minutes at 37° C. To the synthetic surfactant thus formed was admixed 10 μl of 9% NaCl.

Method C was accomplished by first maintaining the polypeptide-PL admixture at 43° C. for 10 minutes, after which time the solvents were removed from the admixture by drying under N_2. When needed, admixtures were further dried by 15 minutes exposure to vacuum to form a dried polypeptide-PL admixture. Water was then admixed with each dried admixture in an amount calculated to equal 90% of the volume necessary to give a final PL concentration of either 5 or 10 mg/ml (as indicated in Table 7) to form a second admixture. This second admixture was maintained for one hour at 43° C. with agitation. Subsequently, a volume of 6% NaCl equal to 10% of the volume necessary to give the desired PL concentration was admixed with the second admixture and the resulting final admixture was maintained for 10 minutes at 43° C. In most cases, the final admixture was subjected to a last step of 3 cycles of freezing and thawing.

B. Results

The synthetic surfactants illustrated in Table 6 were prepared as indicated in the table.

TABLE 6

Peptide	Solvent	(1) Admixture Formed	(2) Phospholipid Admixture Method	(3) Composite Hydrophobicity Value
p1–15	n-propyl alcohol	suspension	A	−16.7
p11–25	H_2O	solution	B	+1.7
p21–35	Chloroform	suspension	A	−9.2
p31–45	H_2O	solution	B	−9.9
p41–55	H_2O	solution	B	−5.4
p51–65	H_2O	suspension	B	−2.2
p61–75	methanol	suspension	A	−9.9
p71–81	H_2O	suspension	B	+3.9
p74–81	H_2O	solution	B	+3.7
p66–81	methanol:H_2O	suspension	A	−1.0
p52–81	methanol:H_2O	suspension	A	−6.2

(1) Each polypeptide was admixed with the indicated solvent to achieve a concentration of 2.5 μg of peptide per μl of solvent.
(2) The letters indicate the synthetic surfactant preparation method used. Those methods are described above.
(3) The composite hydrophobicity value of each peptide was determined as described above.

Each of the synthetic surfactants indicated in Table 6 were assayed for surfactant activity as evidenced by their ability to reduce surface tension in vitro using the "bubble assay" of Enhorning as described above.

The results of this study, shown in FIG. 6, indicate that a subject polypeptide, when admixed with pharmaceutically acceptable phospholipids, forms a synthetic pulmonary surfactant that has greater surfactant activity than the phospholipids alone, as evidenced by the lower ΔP values. Typically 10% to 80% lower ΔP values were obtained using the polypeptides. It should be noted that the 8 amino acid residue control peptide p74–81, which does not conform to the teachings of the present invention, did not form a synthetic PS having a greater activity than the phospholipid alone, thus indicating that amino acid residue length is a critical feature.

The surfactant activity of additional exemplary polypeptides of this invention was studied using the "bubble assay" as described above. The results of the study are illustrated below in Table 7.

Each polypeptide was admixed with the indicated solvent at a concentration of 2.5 mg of polypeptide per ml of solvent. The resulting admixture was observed to determine whether a solution or a suspension of insoluble polypeptide was formed. Those admixtures forming a suspension were further admixed by water bath sonication for 10 seconds to form a very fine suspension, sufficient for pipetting using glass pipettes.

After admixture with solvent, each peptide was admixed with phospholipids (PL), DPPC:PG, 3:1, in chloroform in a glass tube so that the amount of polypeptide added equaled one-tenth (10% by weight) of the amount of PL added, to form a synthetic surfactant according to either method A, B or C.

Each of the synthetic surfactants was then assayed for surfactant activity as evidenced by their ability to reduce surface tension in vitro in the bubble assay performed as described above. The pressure gradient (ΔP) is a measure of surfactant activity in the polypeptide-PL third admixture which was determined using an Enhorning Surfactometer as described above. Measurements were obtained at time points of 15 seconds (15"), 1 minute (1') and 5 minutes (5') and are expressed as a mean of three independent measurements of the indicated polypeptide-PL admixture. Pressure gradient measurements for comparable samples of PL alone (PL) and natural human surfactants were determined as controls.

The result of this study are shown in Table 7.

TABLE 7

Peptide	Solvent	(1) Admixture Formed	(2) Phospholipid Admixture Method	(3) Conc. of PL mg/ml	(4) Pressure Gradient 15"	1'	5'
p1-15	N-propanol	suspension	A	4	0.94	0.82	0.48
p36-81	50% chloroform 50% methanol	suspension	C+	10	0.90	0.87	0.79
p46-76	64% chloroform 36% methanol	solution	C+	10	0.90	0.80	0.62
p51-72	75% chloroform 25% methanol	suspension	C+	10	1.15	0.76	0.33
p51-76	37% chloroform 63% methanol	solution	C+	10	0.99	0.91	0.42
p51-80	45% chloroform 55% methanol	solution	C+	10	0.92	0.89	0.48
p51-81	50% chloroform 50% methanol	suspension	C+	10	0.94	0.86	0.64
p52-81	67% DMF 33% chloroform	solution	A	4	1.33	1.19	0.96
p54-72	76% chloroform 24% methanol	suspension	C+	10	1.28	0.92	0.38
p54-76	71% chloroform 24% methanol	suspension	C+	10	0.92	0.82	0.23
p59-81	68% chloroform 32% methanol	solution	C–	4	1.08	1.02	0.75
p66-81	40% DMF 60% chloroform	suspension	A	4	1.22	1.11	0.84
p74-81	water	solution	B	4	2.37	2.32	2.31
DL4 (31 mer)	47% chloroform 53% methanol	solution	C–	4	2.00	1.80	1.30
RL4	32% chloroform 68% methanol	solution	C–	4	0.58	0.65	0.33
RL8	19% chloroform 81% methanol	suspension	C+	10	0.68	0.69	0.19
RRL7	49% chloroform 51% methanol	solution	C–	4	1.65	1.25	1.00
RCL-1	79% chloroform 21% methanol	suspension	C+	10	0.50	0.59	0.06
RCL-2	67% chloroform 33% methanol	suspension	C+	10	0.00	0.00	0.00
RCL-3	75% chloroform 25% methanol	suspension	C+	10	0.55	0.51	0.33
PL			C+	10	>2.50	>2.50	2.33
Natural Human Surfactant				10	1.06	0.89	0.79

(1) Whether the initial admixture of peptide was a solution or a suspension is indicated.
(2) The letters indicate the synthetic surfactant preparation method used. Those methods are described above. A "+" indicates that the final admixture was subjected to a last step of 3 cycles of freezing and thawing. A "–" indicates the step was not performed.
(3) Concentration ("Conc.") of phospholipid (PL) in the final third admixture is indicated in milligrams PL per milliliter admixture (mg/ml).
(4) The pressure gradient is a measure of surfactant activity in the polypeptide-PL final admixture as determined using an Enborning Surfactometer as described in Example 2. Measurements were obtained at three points of 15 seconds (15"), 1 minute (1') and 5 minutes (5') and are expressed as a mean of 3 independent measurements of the indicated polypeptide-PL admixture. Pressure gradient measurements for comparable samples of PL alone (PL) and natural human surfactant are also shown.

These results indicate that a subject polypeptide, when admixed with pharmaceutically acceptable phospholipids, forms a synthetic pulmonary surfactant that has a greater surfactant activity than the phospholipids alone, as demonstrated by the lower surface pressures obtained.

Example 3

In Vivo Assessment of Synthetic Surfactant Activity

A. Methods

1. Preparation of Synthetic Surfactants

A subject polypeptide was first admixed with solvent as described in Example 2. The resulting admixture was further admixed with phospholipid (PL) so that the amount of polypeptide added was either 3%, 7% or 10% by weight of the amount of PL added as indicated below. The final polypeptide, PL admixture (synthetic surfactant) was formed according to method C using the final freeze thaw step as described in detail in the "Preparation of Synthetic Surfactants" section in Example 2, except that the final admixture had a concentration of 20 mg phospholipid per ml of final admixture.

2. Instillation Protocols

Protocol 1: Fetal rabbits were treated by injection into the trachea of a 0.1 ml solution that contained either a synthetic surfactant prepared in Example 3A or either 2 mg of native surfactant prepared as described in Example 1 or 2 mg PL.

Protocol 2: Synthetic surfactant was instilled in rabbit fetal lung by injection into the trachea from a single syringe of the following three components such that the components exit the syringe in the following order: (1) 0.05 ml air; (2)

0.1 ml of a synthetic surfactant prepared in Example 3A or either 2 mg of PL or 2 mg of native surfactant; and (3) 0.1 ml air.

Protocol 3: From one syringe, a 0.1 ml aliquot of synthetic surfactant prepared as described in Example 3A (or 2 mg of NS or of PL), was instilled into the rabbit trachea as described above, followed by injection of 0.05 ml lactated Ringer's Solution and 0.2 ml air from a second syringe.

Protocol 4: From one syringe, 0.1 ml of a synthetic surfactant prepared as described in Example 3A (or 2 mg of NS or of PL), 0.15 ml air, 0.1 ml saline, and 0.3 ml air were injected into the trachea as described above. Two subsequent aliquots of 0.3 ml air were given.

Protocol 5: Fetal rabbits were treated by injection into the trachea from a single syringe the following four components such that the four components exit the syringe upon injection in the order listed: (1) 0.2 ml solution that contains either a synthetic surfactant prepared in Example 3A or either 4 mg of native surfactant, or 4 mg PL; (2) a 0.15 ml volume of air; (3) a 0.1 ml normal saline solution; and (4) a 0.3 ml volume of air. The above injection was then repeated 15 minutes after the first injection.

Protocol 6: Rabbits were treated as described in Protocol 5, except that two subsequent aliquots of 0.3 ml air were given following the initial instillation and no additional instillation was given at 15 min.

3. Fetal Rabbit Model for Studying Surfactant Activity

The surfactant activity of exemplary polypeptides of this invention was studied using the methods described in detail previously by Revak, et al. *Am. Rev. Respir. Dis.* 134: 1258–1256 (1986), with the exceptions noted hereinbelow.

Twenty-seven day gestation fetal rabbits were delivered by hysterotomy and immediately injected with 0.05 ml Norcuron (Organon, Inc., N.J.) to prevent spontaneous breathing. The fetal rabbits were then weighed and a small cannula was inserted into the trachea by tracheotomy. Synthetic surfactant prepared as described above was then instilled into the fetal rabbit lung by one of the above instillation protocols.

Following instillation the rabbit was placed in a specially designed plethysmograph (containing a Celesco transducer) connected to a ventilator (Baby Bird, Baby Bird Corp., Palm Springs, Calif.) and the instilled lung was ventilated at a rate of 30 cycles per minute with a peak inspiratory pressure of 25 cm H_2O, a positive end expiratory pressure of 4 cm H_2O and an inspiratory time of 0.5 seconds. In some studies, dynamic compliance measurements were made at various times throughout the ventilation procedure. In others, static compliance measurements were made following ventilation.

Static compliance measurements were made after 30 minutes of ventilation. The animals were removed from the ventilator and the lungs were degassed at −20 cm H_2O in a bell jar under vacuum. Thereafter, the lungs were first inflated and then deflated through a T-connector attached to a tracheostomy tube. The volume of air required to reach static pressures of 5, 10, 15, 20, 25 and 30 cm H_2O was measured during both inflation and deflation phases to generate static pressure to volume curves as a measure of static compliance.

Using the plethysmograph, dynamic compliance measurements were made at various times throughout a 60 minute ventilation period. Computer-assisted data analysis resulted in compliance data expressed as ml of air per cm H_2O per gram of body weight at each time point. Compliance was calculated by the formula below.

$$\text{Compliance} = \frac{\Delta V}{\Delta P}$$

$\Delta P_{tp}=(C)^{-1}\cdot(\Delta V)+(R)\cdot(F)$

P_{tp}=transpulmonary pressure

C=compliance (elastic component—relates change in volume to pressure)

R=resistance (relates flow to pressure)

F=flow

V=volume=the integral of flow with respect to time

The above equation was solved with a multiple linear regression for C and R. The compliance (C) represents the elastic nature of the lungs and the resistance (R) represents the pressure necessary to overcome the resistance to the flow of gas into and out of the lungs.

B. Results

Static compliance data using instillation protocols 1 and 5 are shown in FIGS. 7 and 8, respectively. Improved lung compliance was seen in all lungs treated with natural surfactant or with the synthetic surfactants tested as compared with those lungs treated with phospholipids (PL) alone, with one exception. The synthetic surfactant prepared using p1–15 (FIGS. 8A and 8E) did not produce improved lung compliance over PL alone when measured by static compliance.

The results of the dynamic compliance studies are illustrated in Table 8.

TABLE 8

		Dynamic Compliance in ml air/cm H_2O (g body weight × 10^6)						
	% Peptide Compound	Minutes after Surfactant Instillation						Sample[1] Given By Protocol
PL	To PL	10	20	30	40	50	60	#
PL		7	8	7	10	11	15	4
		24	22	23	23	22	20	4
		15	16	17	18	21	29	4
NS		265	251	168	186	173	147*	4
		418	388	405	288	237	*	4
		155	176	172		172	179	4
p36-81	5%			255			146*	3
	5%			245			291	3
	10%			154			1,162	2
	10%			252			623	2
p52-81	5%			517			226*	3
	5%			434			55*	3
	10%			195			1,243	2
	10%			43			1,690	2
p51-76	10%	33	22	56	87	124	85	4
	10%	10	11	186	358	141	144*	4
	10%	15	36	109	241	264	301	4
p51-80	10%	17	41	52	78	99	208	4
	10%	76	94	149	149	217	308	4
	10%	23	71	130	156	182	109*	4

[1]Prior to instillation into the rabbits, these samples were filtered through a 25 micron filter.

*A decrease in compliance with time may indicate the development of pneumothorax.

As shown in Table 8, each of the synthetic surfactants of this invention and natural surfactant improved dynamic compliance values in comparison to phospholipid alone.

C. Discussion

The in vivo compliance studies demonstrate that the use of a number of exemplary synthetic surfactants of this inventions resulted in enhanced compliance in comparison

to phospholipid alone for each of the assayed synthetic surfactants. Thus, the proteins and polypeptides of this invention when admixed with pharmaceutically acceptable phospholipids form synthetic surfactants that have greater surfactant activity than phospholipid alone. Use of the synthetic surfactants is advantageous in producing improved compliance values in vivo.

Example 4

Study of Binding of C-Terminal Peptide to Lung Epithelial Cells

A. Methods—Peptide Binding Assay

A peptide having residues 74–80 of SP18 (VLRCSMD) was radiolabeled by the Bolton-Hunter method (Bolton et al., *Biochem J.* 133: 529–538 1973) with ^{125}I (New England Nuclear—34.1 moles/ml, 28.0 ng/ml, 75 μCi/ml).

Human pulmonary epithelial cells (human lung carcinoma cell. ATCC reference no. CCL 185, commonly known as A549 cells) were grown to confluence in 6 well tissue culture dishes. The following solutions were used in this study:

PBS/BSA: 10 mM Na Phosphate+0.15M NaCl+0.5% BSA pH 7.4

Lysis Buffer: 1% SDS in water

Solution F: 5 ml PBS/BSA+51.56 μg cold peptide

Solution D: 2.5 ml PBS/BSA+87 μl ^{125}I-peptide

Solution D1/5: 0.5 ml D+2.0 ml PBS/BSA

Solution D/125: 0.5 ml D1/5+2.0 ml PBS/BSA

Solution E: 2.5 ml PBS/BSA+87 μ^{125}I-peptide +20.78 μg cold peptide

Solution E1/5: 0.5 ml E 2.0 ml PBS/BSA

Solution E 1/25: 0.5 ml E1/5+2.0 ml PBS/BSA

Three 6-well plates were pretreated by incubating with 0.5 ml of the following solutions for 15 min. at 22° C. The odd-numbered wells were pretreated with PBS/BSA and the even-numbered wells with solution F. Following removal of the pretreatment solution, the wells were incubated with 0.5 ml of the following solutions at 22° C. for the indicated time while gently rocking the plates.

Well	Sample	Incubation Time
1	D	7 minutes
2	E	7 minutes
3	D 1/5	7 minutes
4	E 1/5	7 minutes
5	D 1/25	7 minutes
6	E 1/25	7 minutes
7	D	30 minutes
8	E	30 minutes
9	D 1/5	30 minutes
10	E 1/5	30 minutes
11	D 1/25	30 minutes
12	E 1/25	30 minutes
13	D	143 minutes
14	E	143 minutes
15	D 1/5	143 minutes
16	E 1/5	143 minutes
17	D 1/25	143 minutes
18	E 1/25	143 minutes

At the end of the incubation time the supernatant was removed from each well and saved for counting. Each well was washed four times with cold (4° C.) PBS/BSA. The washes were saved for counting. The plate was then brought back to room temperature and 1 ml of lysis buffer was added to each well. The plate was gently shaken until all cells had lysed and come off the plate (3–4 minutes). The solution was removed from each well and counted. A second ml of lysis buffer was added to each well, mixed a few minutes and removed for counting of bound counts. The percent and absolute amounts of counts bound were determined.

Specific counts bound were determined by subtracting the counts bound in wells containing unlabeled (cold) peptide from the corresponding well without cold peptide. The results are illustrated in Table 9, below.

The procedure was repeated with the following changes:

D_1=1433 μl PBS/SA+167 μl ^{125}I-peptide |1.78 pmol/500 μ|

D_2=183.3 μl PBS/BSA+366.7 μl D_1 |1.19 pmol/500 μl|

D_3=275 μl BPS/BSA 275 μl D_1 |0.89 pmol/500 μ|

D_4=366.7 μl BPS/BSA+183.3 μl D_1 |0.59 pmol/500 μ|

D_5=458.3 μl BPS/BSA+91.7 μl D_1 |0.30 pmol/500 μ|

D_6=513.3 μl BPS/BSA+36.7 μl D_1 |0.12 pmol/500 μ|

E_1=1386.24 μl BPS/BSA+167 μl ^{125}I-peptide |4.676 μg|+ 46.76 μl cold peptide at 100 μg/ml |4.1676 μ|

E_2–E_6 Diluted as above for D_2–D_6.

F=3.398 Ml PBS/BSA+102.29 μl cold peptide at 100 μg/ml

Two six-well plates were washed once with 1 ml PBS/BSA. The odd numbered-wells were pretreated with PBS/BSA and the even-numbered wells with solution F. Following removal of the pretreatment solution, the following solutions were added:

Well	Sample	Well	Sample
1	D_1	7	D_4
2	E_1	8	E_4
3	D_2	9	D_5
4	E_2	10	E_5
5	D_3	11	D_6
6	E_3	12	E_6

The solutions were incubated for 30 minutes at room temperature with gentle rocking. The supernatants were then removed and saved for counting. Each well was washed 4 times with 0.5 ml of cold PBS/BSA. Washes were saved for counting. 1 ml of 1% SDS was added to each well to solubilize the cells. After 3 minutes all the cells could be seen to have come off the plate. The lysed cell-containing supernatant was counted, together with a second SDS wash of the wells. Total counts and the percentage of counts bound were determined. Specific binding was determined by subtracting the counts bound in wells containing cold peptide from the corresponding well without cold peptide. The results are illustrated in Table 10.

B. Results

The results of the binding studies are illustrated below in Tables 9 and 10.

TABLE 9

Well	Total CPM	Total CPM Bound	% Bound	Specific Difference	Cts Bound*
1	1,109,126	24,414	2.23		
2	1,087,659	17,353	1.60	0.63%	6,930
3	223,170	4,479	2.01		
4	221,608	4,113	1.86	0.15%	330
5	45,877	828	1.80		

TABLE 9-continued

Well	Total CPM	Total CPM Bound	% Bound	Specific Difference	Cts Bound*
6	47,731	880	1.84	−0.04%	−18
7	1,103,606	25,905	2.35		
8	1,152,287	19,230	1.67	0.68%	7,480
9	227,396	4,996	2.20		
10	230,974	4,137	1.79	0.41%	901
11	47,899	1,030	2.15		
12	49,894	922	1.85	0.30%	132
13	1,151,347	10,071	.87		
14	1,108,755	9,506	.86	0.01%	110
15	220,340	1,692	.77		
16	229,253	1,800	.79	−0.02%	−44
17	46,893	407	.87		
18	47,426	386	.81	0.06%	26

*Corrected to 1,100,000 cpm/undiluted tube.

TABLE 10

Well	Total CPM	CPM Bound	% Bound	Corrected Difference	p mol
1	3,070,705	66,954	2.78		
2	2,995,775	56,055	1.87	9,390	1.78
3	2,029,323	39,562	1.95		
4	2,013,557	33,573	1.67	5,723	1.19
5	1,436,189	26,883	1.87		
6	1,427,731	25,073	1.76	1,755	.89
7	994,288	15,669	158		
8	964,481	14,776	1.53	503	.59
9	460,317	6,513	1.41		

TABLE 10-continued

Well	Total CPM	CPM Bound	% Bound	Corrected Difference	p mol
10	479,746	6,816	1.42	−52	.30
11	202,494	2,930	1.45		
12	192,990	2,806	1.45	0	.12

*Corrected for 1.78 p mol = 3,033,740 cpm

C. Discussion

As can be seen from the data in Table 9, the study demonstrated that the peptide was binding specifically to the cells as demonstrated by competitive inhibition by unlabeled peptide. However, the cells were not saturated by the amount of labeled peptide used in this study. Additionally, degradation of the peptide was occurring by 143 minutes.

The second study was performed using the 30 minute incubation period and an increased amount of labeled peptide to achieve saturation of the cells. As seen in Table 10, specific binding was again demonstrated. Further, saturation was achieved as demonstrated by leveling-off of the amount of bound counts at high concentration of labeled peptide.

The binding studies thus demonstrate that the C-terminal peptide of this invention binds specifically to lung epithelial cells.

The foregoing specification, including the specific embodiments and examples, is intended to be illustrative of the present invention and is not to be taken as limiting. Numerous other variations and modifications can be effected without departing from the true spirit and scope of the present invention.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i i i) NUMBER OF SEQUENCES: 3

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: peptide

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Lys Leu Leu Leu Leu Lys Leu Leu Leu Leu Lys Leu Leu Leu Leu Lys
1 5 10 15

Leu Leu Leu Leu Lys
20

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: peptide

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Lys Leu Leu Leu Leu Leu Leu Leu Leu Lys Leu Leu Leu Leu Leu Leu
1 5 10 15

-continued

Leu Leu Lys Leu Leu
20

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: peptide

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Lys Lys Leu Leu Leu Leu Leu Leu Leu Lys Lys Leu Leu Leu Leu Leu
1 5 10 15

Leu Leu Lys Lys Leu
20

We claim:

1. A pulmonary surfactant comprising one or more pharmaceutically acceptable phospholipids admixed with a polypeptide having an amino acid residue sequence represented by the formula KLLLLKLLLLKLLLLKLLLLK (SEQ. ID NO: 1), said polypeptide, thereby forming a pulmonary surfactant having a surfactant activity greater than the surfactant activity of the phospholipid alone, said phospholipid being present in the range of about 50–100 weight percent, in a polypeptide:phospholipid weight ratio in the range of about 1:7 to about 1:1,000.

2. The pulmonary surfactant of claim 1, wherein said phospholipid is selected from the group consisting of:

1,2-dipalmitoyl-sn-glycero-3-phosphocholine (dipalmitoylphosphatidylcholine, DPPC);

phosphatidyl glycerol (PG); and

an admixture of DPPC and PG in a weight ratio of about 3:1.

3. The pulmonary surfactant of claim 1, further comprising palmitic acid, wherein said phospholipid comprises about 50–90 weight percent and said palmitic acid comprises the remaining 10–50 weight percent of said surfactant.

4. A method of treating respiratory distress syndrome comprising administering a therapeutically effective amount of a pulmonary surfactant, said surfactant comprising one or more pharmaceutically acceptable phospholipids admixed with a polypeptide having an amino acid residue sequence represented by the formula KLLLLKLLLLKLLLLKLLLLK (SEQ. ID NO: 1), said polypeptide, when admixed with a pharmaceutically acceptable phospholipid, forming a pulmonary surfactant having a surfactant activity greater than the surfactant activity of the phospholipid alone, said phospholipid being present in the range of about 50–100 weight percent, in a polypeptide:phospholipid weight ratio in the range of about 1:7 to about 1:1,000, or in an amount such that it may be administered in a range of about 50 mg/kg to about 500 mg/kg per dose.

5. The method of claim 4, wherein said phospholipid is selected from the group consisting of:

1,2-dipalmitoyl-sn-glycero-3-phosphocholine (dipalmitoylphosphatidylcholine, DPPC);

phosphatidyl glycerol (PG); and

an admixture of DPPC and PG in a weight ratio of about 3:1.

6. The method of claim 4, wherein said surfactant further comprises palmitic acid, and wherein said phospholipid comprises about 50–90 weight percent and said palmitic acid comprises the remaining 10–50 weight percent of the lipid portion of said surfactant.

* * * * *

Exhibit E

[redacted]

[redacted]

United States Patent and Trademark Office

Patent Bibliographic Data				09/04/2009 09:37 AM
Patent Number:	5789381	**Application Number:**	08419824	
Issue Date:	08/04/1998	**Filing Date:**	04/11/1995	
Title:	PULMONARY SURFACTANT PROTEINS AND RELATED POLYPEPTIDES			
Status:	12th year fee window opens: 08/04/2009		**Entity:**	Large
Window Opens:	08/04/2009	**Surcharge Date:**	02/05/2010	**Expiration:** N/A
Fee Amt Due:	$4,110.00	**Surchg Amt Due:**	$0.00	**Total Amt Due:** $4,110.00
Fee Code:	1553	MAINTENANCE FEE DUE AT 11.5 YEARS		
Surcharge Fee Code:				
Most recent events (up to 7):	01/13/2006 01/10/2002 06/24/1997	Payment of Maintenance Fee, 8th Year, Large Entity. Payment of Maintenance Fee, 4th Year, Large Entity. Payor Number Assigned. --- End of Maintenance History ---		
Address for fee purposes:	Olson & Cepuritis, LTD. 20 NORTH WACKER DRIVE 36TH FLOOR CHICAGO, IL 60606			

NOTE: All USPTO fees are subject to change. If you are making a payment by mail or fax, please visit this link or contact the Maintenance Fee Branch (571-272-6500) to confirm the amount due on the date payment is to be made. A maintenance fee payment can be timely made using the certificate of mailing or transmission procedure set forth in 37 CFR 1.8.

[redacted]

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Exhibit F

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 5,789,381
DATED : August 4, 1998
INVENTOR(S) : Cochrane et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 1,
Line 4, insert: -- This invention was made with government support under Grant Nos. HL. 23584 and GM 37696 from the National Institutes of Health and Grant No. N00014-89-K-0029 from the Office of Naval Research. The U.S. government may have certain rights in the invention. --

Signed and Sealed this

Ninth Day of April, 2002

Attest:

Attesting Officer

JAMES E. ROGAN
Director of the United States Patent and Trademark Office

Exhibit G

TERMINAL DISCLAIMER TO OBVIATE A DOUBLE PATENTING REJECTION OVER A PRIOR PATENT	Docket Number (Optional) TSRI-147.2 Con 2

In re Application of: Charles G. Cochrane and Susan D. Revak

Application No. 08/419,824

Filed: April 11, 1995

For: PULMONARY SURFACTANT PROTEINS AND RELATED POLYPEPTIDES

The owner, The Scripps Research Institute, of 100 percent interest in the instant application, hereby disclaims, except as provided below, the terminal part of the statutory term of any patent granted on the instant application, which would extend beyond the expiration date of the full statutory term defined in 35 U.S.C. 154 to 156 and 173, as presently shortened by any terminal disclaimer, of prior Patent Nos. 5,260,273 and 5,407,914. The owner hereby agrees that any patent so granted on the instant application shall be enforceable only for and during such period that it and the prior patent are commonly owned. This agreement runs with any patent granted on the instant application and is binding upon the grantee, its successors or assigns.

In making the above disclaimer, the owner does not disclaim the terminal part of any patent granted on the instant application that would extend to the expiration date of the full statutory term as defined in 35 U.S.C. 154 to 156 and 173 of the prior patent, as presently shortened by any terminal disclaimer, in the event that it later: expires for failure to pay a maintenance fee, is held unenforceable, is found invalid by a court of competent jurisdiction, is statutorily disclaimed in whole or terminally disclaimed under 37 CFR 1.321, has all claims cancelled by a reexamination certificate, is reissued, or is in any manner terminated prior to the expiration of its full statutory term as presently shortened by any terminal disclaimer.

Check either box 1 or 2 below, if appropriate.

1. ( ) For submissions on behalf of an organization (e.g., corporation, partnership, university, government agency, etc.), the undersigned (whose title is supplied below) is empowered to act on behalf of the organization.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

2. (x) The undersigned is an attorney of record.

12 September 1997
Date

Talivaldis Cepuritis
Signature

Talivaldis Cepuritis (Reg. No. 20,818)
Typed or printed name and title if applicable

(x) Terminal disclaimer fee under 37 CFR 1.20(d) included.

(x) PTO suggested wording for terminal disclaimer was
(x) unchanged, ( ) changed (if changed. an explanation should be supplied).

TERMINAL DISCLAIMER TO OBVIATE A DOUBLE PATENTING REJECTION OVER A PRIOR PATENT	Docket Number (Optional) TSRI 147.2 Con.2

In re Application of: Charles G. Cochrane et al.

Application No. 08/419,824

Filed: April 11, 1995

For: PULMONARY SURFACTANT PROTEINS AND RELATED POLYPEPTIDES

The owner, The Scripps Research Institute, of 100 percent interest in the instant application, hereby disclaims, except as provided below, the terminal part of the statutory term of any patent granted on the instant application, which would extend beyond the expiration date of the full statutory term defined in 35 U.S.C. 154 to 156 and 173, as presently shortened by any terminal disclaimer, of prior Patent No. 5,164,369. The owner hereby agrees that any patent so granted on the instant application shall be enforceable only for and during such period that it and the prior patent are commonly owned. This agreement runs with any patent granted on the instant application and is binding upon the grantee, its successors or assigns.

In making the above disclaimer, the owner does not disclaim the terminal part of any patent granted on the instant application that would extend to the expiration date of the full statutory term as defined in 35 U.S.C. 154 to 156 and 173 of the prior patent, as presently shortened by any terminal disclaimer, in the event that it later: expires for failure to pay a maintenance fee, is held unenforceable, is found invalid by a court of competent jurisdiction, is statutorily disclaimed in whole or terminally disclaimed under 37 CFR 1.321, has all claims cancelled by a reexamination certificate, is reissued, or is in any manner terminated prior to the expiration of its full statutory term as presently shortened by any terminal disclaimer.

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2. (x) The undersigned is an attorney of record.

4 December 1997 Talivaldis Cepuritis

Date Signature

Talivaldis Cepuritis (Reg. No. 20,818)

Typed or printed name and title if applicable

(x) Terminal disclaimer fee under 37 CFR 1.20(d) included.

(x) PTO suggested wording for terminal disclaimer was

(x) unchanged, ( ) changed (if changed. an explanation should be supplied).

Exhibit H

SURFAXIN® US IND #40,287 Brief Description of Significant Events

Date	*Type of Document*	*Description*
7/31/1992	Original Submission	Scripps Institute (Sponsor Charles G. Cochrane, MD) filed IND for SURFAXIN with the FDA under Section 505(i) of the Federal Food, Drug and Cosmetic Act and 21 CFR §312.
8/6/1992		The date of receipt of IND by the FDA.
8/11/1992	FDA Letter	Acknowledgement of receipt of IND Application.
8/25/1992	Scripps Letter	Suggested Modifications in the Clinical Protocol Pursuant to Discussions with Dr. Himmel at FDA.
8/31/1992	Scripps Letter	Letter to Dr. Himmel from Dr. Cochrane adding information to provide supporting data for dosage form.
9/1/1992	FDA Letter	Additional comments of safety and consent form from Dr. Himmel.
9/3/1992	Scripps Letter	Resulting changes per Dr. Himmel's suggestions in letter dated 9/1/92.
9/10/1992	Scripps Letter	Revised Patient Consent Form for Protocol.
9/11/1992	FDA TELEFAX	Fax from FDA regarding the Patient Consent Forms.
9/25/1992	Scripps Letter	Revised Patient Consent Forms with revisions according to the requested FDA guidelines in your FAX of 9/11/92.
1/8/1993	Scripps Letter	Final Consent Forms from the 3 University of California campuses.
3/17/1003	Scripps Letter	Expansion of weight range request.
3/19/1993	Scripps Letter	Addendum for drug dose.
4/19/1993	FDA Letter	Comments on preclinical studies re 1/8/93 amendment.
5/19/1993	Scripps Letter	Reply to 4/19/93 FDA letter re: IND # 40,287.
6/30/1993	Protocol Amendment: Change in Protocol Information Amendment: Chemistry/Microbiology Information Amendment: Pharmacology/Toxicology	Amendment to CP 1 protocol , (CP 1 to be referred to as CP 2) Introductory Statement, Investigator's Brochure, Protocol, CMC, Pharm/Tox, and Previous Human Experience. Minor changes to investigator's brochure. Protocol CP-2 (high dose). CMC changes to higher dose.
8/4/1993	Scripps Letter	Minor protocol change to Amendment # 001 (6/30/93)
9/7/1993	FDA Letter	Comments on Amendment 6/30/93. FDA is continuing to review this amendment, however, they see no objectionable data to increasing the dose. Recommends that subjects are randomized to the two doses so that dose response data can be obtained.
9/21/1993	Protocol Amendment: New Investigator	Added four (4) new investigators to CP2 Protocol. Attachments: relevant form FDA 1572s and CVs; Copy of ICF from each new sites
10/27/1993	Scripps Letter	Proposed changes to Amendment 001.
10/29/1993	Scripps Letter	Scripps grants R.W Johnson Pharmaceutical Research Institute (RWJPRI) permission to send direct communications to Scripps IND #40,287.
11/2/1993	Annual Report	1993 Annual Report
11/5/1993	Scripps Letter	Submission of pre-clinical protocol TSRI # 94-1 for FDA review.
11/18/1993	FDA TELEFAX	Comments from complete medical review from 10/27/1993 amendment
11/18/1993	FDA Letter	Review of Scripps Letter dated 10/27/93.
11/19/1993	FDA Letter	Comments from FDA on submission dated 11/5/93.
11/19/1993	Scripps Letter	Letter to confirm receipt of changes to protocol for newborn rabbit studies.
12/29/1993	Scripps Letter	Himmel's request for information on high frequency ventilation in infants with RDS receiving surfactant.
9/21/1994	Annual Report	1994 Annual Report.
4/13/1995	Scripps Letter	Request End of Phase II Conference.

Date	*Type of Document*	*Description*
5/19/1995	Scripps Letter	Partial transfer of sponsor responsibilities from Scripps to Beardsworth Consulting Group.
5/19/1995	General Correspondence: Response to FDA Request for Information	Response to FDA Request for Information. Proposed agenda for End of Phase II conference; Letter of Authorization to cross-reference PRI IND 46,164; Clinical Data Summary from CP1 and CP2; Listing of proposed Phase 2 Clinical Sites; CP-3 Protocol (Draft #4, 5/17/95); Brief summary of CP4; Summary of CM&C changes; Summary of Pharmacology/toxicology.
7/20/1995	Scripps Letter	Information obtained from the Orphan Drug application.
8/23/1995	General Correspondence: Pre-Meeting Package: End of Phase II Meeting	Revised Clinical Protocols (KL4-IRDS-001and -002); Revised Table 10 of Clinical Data Summary from Phase I/II; Updated Toxicology Tables and Summary of Planned Multiple Dose Rat Study; Summary of Planned Drug Metabolism Studies; Summary of Planned Preclinical Pharmacology Studies; Updated Proposed Agenda; List of attendees for 09/11/1995 End of Phase II RDS Meeting.
8/24/1995	Information Amendment: Chemistry/Microbiology Information Amendment: Clinical Information Amendment: Pharmacology/Toxicology	New Investigator's Brochure; Cross-reference to CM&C in 46,164. Pharm/Tox- overview, summary tables, summaries of pre-clinical studies filed to 46,164; Filing of TSRI 94-1; Updated human experience.
10/10/1995	Scripps Letter	Summary of 9/11/95 End of Phase II Meeting.
10/20/1995	Annual Report	1995 Annual Report.
10/31/1996	Annual Report	1996 Annual Report.
12/4/1996	General Correspondence: Transfer of Sponsorship	Transfer of Sponsorship from Scripps to Acute Therapeutics, Inc. (ATI).
12/5/1996	General Correspondence: Acceptance of Ownership	Acceptance of Ownership and Responsibility by ATI.
12/13/1996	FDA TELEFAX	Meeting minutes from the FDA. Meeting was held to discuss the proposed study design for Protocol KL4-IRDS-001 as well as other issues pertinent to an NDA Submission.
12/18/1996	FDA Letter	Acceptance of Transfer of 40,287 to Acute Therapeutics, Inc.
3/25/1997	Information Amendment: Pharmacology/Toxicology	Pharm/Tox: Includes 3 final reports ; Mass Balance Determination of 3H-KL4 Surf. In Rats Following A Single Intratracheal Dose of 3H-KL4 Surf; Acute Intratracheal Safety of RWJ-45652-021 in Crl:CDBR, VAF/Plus Rats; 7 Day Intratracheal Toxicity Study of RWJ 45652-021.
3/27/1997	FDA TELEFAX	FDA's 3/10/97 meeting minutes.
4/17/1997	Information Amendment: Chemistry/Microbiology	Revised CMC section of IND 40,287 I: Introduction, II: Drug Substance, III: Other active ingredients, IV: Drug Product, V: Environmental Assessment.
6/6/1997	Information Amendment: Clinical	Curriculum Vitae of new Medical Monitor for Discovery Laboratories, Inc.
7/3/1997	Information Amendment: Chemistry/Microbiology	Two-month Stability Reports for SURF-0001 & SURF- 0002.
9/30/1997	Annual Report	1997 Annual Safety Report. New Information Included: Preclinical Study Information and Manufacturing and/or Microbiological Changes.
1/14/1998	Information Amendment: Chemistry/Microbiology	Amended CMC section to correct an inadvertent error in the container-closure system information for the finished drug substance.
3/31/1998	Information Amendment: Chemistry/Microbiology	PCD Report (PCD-98-002) on the validity of the Dynamic Surface Tension Assay. Revised Surfaxin stability protocol.

Date	*Type of Document*	*Description*
6/29/1998	General Correspondence: Change in Company Name	Change in company name from Acute Therapeutics Inc. to Discovery Laboratories Inc.
9/8/1998	Information Amendment: Clinical	Full clinical report for protocols CP-1 and CP-2.
10/30/1998	Annual Report	Annual Safety Report for the period August 1, 1997 to July 31, 1998.
6/4/1999	DSCO Telefax	Follow-up to 6/4/99 telecon regarding FDA comments on proposed phase 3 equivalency trial in RDS. Fax provided protocol concept sheet and Position Paper
7/2/1999	Information Amendment: Clinical	Protocol concept sheet for Surfaxin/Survanta equivalency trial and a supporting position paper.
8/12/1999	General Correspondence: Request for End-of-Phase 2 Meeting	End of Phase 2 meeting agenda, discussion items, introduction, preclinical overview, clinical overview and CMC overview.
8/19/1999	FDA TELEFAX	Comments on proposed Phase 3 equivalency trial in RDS.
8/19/1999	FDA Letter	Comments regarding the proposed Phase 3 protocol for RDS.
9/3/1999	DSCO Telefax	Discussion items for the September 8, 1999 Phase 3 IRDS trial teleconference
9/10/1999	DSCO Telefax	Proposed equivalency prevention study comparing Surfaxin and Survanta; Discovery's minutes from the September 8, 1999 teleconference
9/10/1999	DSCO Telefax	1999 IRDS teleconference
9/27/1999	FDA TELEFAX	List of FDA attendees for the 10/14/99 End-of-Phase 2 meeting.
11/3/1999	FDA TELEFAX	FDA Comments on End-of-Phase 2 Meeting Package
11/3/1999	Annual Report	Annual Safety Report for the period August 1, 1998 to July 31, 1999.
11/12/1999	General Correspondence: Response to FDA	10/14/99 end-of-phase 2 Discovery meeting minutes.
12/9/1999	FDA Correspondence	FDA comments regarding Discovery's September 10, 1999 meeting minutes from September 9, 1999 telecon.
1/6/2000	FDA TELEFAX	FDA Comments official comments on Discovery meeting minutes from Discovery/FDA teleconference date 9/9/99.
1/12/2000	FDA TELEFAX	FDA agenda for 1/14/00 meeting.
1/12/2000	FDA TELEFAX	Comments on proposed Phase 3 RDS protocol concept.
1/12/2000	DSCO Telefax	Proposed Phase 3 IRDS protocol concept.
1/24/2000	FDA TELEFAX	FDA provided Discovery's meeting minutes revised which were now the agency's official minutes. In addition, they provided several CMC comments.
2/3/2000	General Correspondence	Copy of Discovery meeting minutes from January 14, 2000 RDS Meeting at the FDA. Copy of Statistical Presentation given by Dr. Tsai
2/8/2000	General Correspondence: RDS Phase 3 Clinical Trial Proposal	Revised RDS protocol concept sheet; Study design rationale paper.
3/1/2000	FDA TELEFAX	FDA comments regarding the revised RDS protocol concept sheet for a Surfaxin-Survanta superiority trial (Fax cover page dated 02/28/2000, Fax banner 03/01/2000).
3/1/2000	FDA TELEFAX	FDA meeting minutes from January 14, 2000 meeting regarding the proposed RDS equivalency prevention study comparing Surfaxin with Survanta.
3/3/2000	DSCO Telefax	Discovery's response to the agency's comments regarding the phase 3 protocol concept sheet and rationale.

Date	*Type of Document*	*Description*
3/8/2000	DSCO Telefax	Discovery's response to the agency's request for information and Discovery's meeting minutes from the 3/6/00 teleconference regarding the proposed phase 3 RDS protocol concept sheet and rationale.
3/13/2000	General Correspondence: Response to FDA General Correspondence: Development/Meeting Min	Response to the Agency's comments in the 3/1/00 faxed letter; a copy of the faxed letter; the Discovery meeting minutes from the 3/6/00 teleconference regarding phase 3 RDS concept sheet submitted 2/8/2000; and Discovery's response to the Agency's request for information.
3/28/2000	DSCO Telefax	Discovery alternate RDS protocol proposal.
3/31/2000	DSCO Telefax	Discovery meeting minutes from 3/29/00 teleconference regarding the composite primary endpoints proposed in the phase 3 RDS protocol concept sheet submitted 02/08/2000 serial no 049.
4/4/2000	General Correspondence: Development/Meeting Minutes	Discovery's alternative RDS non-inferiority proposal dated 03/28/2000; Discovery's meeting minutes from the 3/29/00 teleconference regarding composite primary endpoints proposed in the Phase 3 RDS protocol concept sheet.
4/7/2000	DSCO Telefax	Supplement to noninferiorty Surfaxin vs Survanta RDS protocol proposal.
4/14/2000	FDA TELEFAX	Agency meeting minutes from the 3/6/00 teleconference regarding DCSO responses to agency comments on submitted phase 3 RDS protocol concept sheet.
4/18/2000	FDA TELEFAX	Agency concerns about the current proposal for non-inferiority trial of prophylaxis Surfaxin vs rescue Survanta in the treatment of RDS
5/23/2000	FDA TELEFAX	April 14, 2000 Teleconference minutes regarding the agency concerns for the proposed non-inferiority study
5/26/2000	Information Amendment: Chemistry/Microbiology	CMC Amendment which includes changes in support of the MAS and IRDS indications.
5/26/2000	General Correspondence Information Amendment: Pharmacology/Toxicology	Minutes from April 14, 2000 teleconference; Pharm/tox study.
8/15/2000	Information Amendment: Clinical	Curriculum Vitae of Robert Segal, M.D., new Medical Monitor for Discovery Laboratories, Inc.
8/16/2000	General Correspondence	August 9, 2000 Teleconference meeting minutes regarding performing a RDS placebo controlled trial.
8/30/2000	FDA TELEFAX	Agency telecon meeting minutes for the 8/9/00 teleconference regarding the proposed RDS trial..
10/12/2000	FDA TELEFAX	Revised version of agency meeting minutes from 8/9/00 teleconference regarding the proposed RDS study..
11/13/2000	Protocol Amendment: New Protocol	Protocol KL4-IRDS-04 dated 11/10/2000; Discovery minutes from 8/9/00 teleconference regarding the feasibility of conducting a placebo-controlled RDS trial in Latin America.
11/27/2000	FDA TELEFAX	Letter from agency regarding ethical and data applicability issues with the regard to the proposed phase 3 RDS study.(Received via fax).
11/27/2000	FDA Letter	Letter from agency regarding ethical and data applicability issues with the regard to the proposed phase 3 RDS study. (original FDA letter received 12/4/2000)
12/14/2000	DSCO Telefax	Justification as to why the planned phase 3 RDS study is ethical and an appropriate trial design to support an U.S. marketing application.
1/11/2001	FDA TELEFAX	Agency meeting minutes from 12/18/00 teleconference regarding Discovery's position with respect to the ethicality and data applicability of the proposed phase 3 RDS trial design
1/17/2001	Annual Report	2000 Annual Safety Report for the period August 1, 1999 to July 31, 2000.

Date	*Type of Document*	*Description*
1/18/2001	Protocol Amendment: Change in Protocol General Correspondence: Response to FDA	Response to Agency from 12/18/00 telecon; Copy of Agency's 12/18/00 meeting minutes; Copy of DSCO 12/18/00 meeting minutes regarding ethicality/approvability of proposed phase 3 RDS; Change doc for protocol KL4-IRDS-04 Amend 1; Protocol KL4-IRDS-04 Amend 1; Training syllabus; Synopsis of proposed European Phase 3 RDS study; A copy of Discovery's meeting minutes from the 12/11/00 meeting with the EMEA.
1/26/2001	Information Amendment: Chemistry/Microbiology	CMC Amendment which includes information in support of the MAS and IRDS indications.
2/20/2001	Information Amendment: Clinical	Updated Investigator Brochure dated February 6, 2001.
2/22/2001	DSCO Telefax	Fax to Dr. Myer, follow up to telecon on 2/22/01. Copy of letter sent to Secretary Thompson by the Public Citizen, and notations on the fact that Dr. Sidney Wolfe and cast had a copy of Birenbaums presentation.
2/26/2001	Protocol Amendment: New Protocol Information Amendment: Clinical	Protocol KL4-IRDS-02; KL4-IRDS-02 sample CRF; KL4-IRDS-02 sample ICF; DSCO's 12/14/00 facsimile transmission; KL4-IRDS-04 CRF; KL4- IRDS-04 sample ICF.
3/8/2001	DSCO Telefax	Pre-meeting package for 3-12-01 meeting with the FDA: Provided 1. Proposed Discovery meeting attendees; 2. Proposed agenda; 3. Introduction and background; and 4. An alternate RDS trial design
3/8/2001	General Correspondence: March 12, 2001 Pre-Meeting Package	Proposed Discovery meeting attendees; Proposed meeting agenda; Introduction and background; An alternate trial design.
3/9/2001	FDA TELEFAX	FDA telefax providing list of attendees for 3-12-01 meeting; a proposed agenda; and comments regarding the proposed placebo-controlled trial in Latin America.
3/9/2001	FDA Letter	Formal letter from agency detailing comments regarding KL4-IRDS-04, placebo-controlled RDS study planned for Latin America
3/14/2001	DSCO Telefax	Follow-up from 3-12-01 meeting. Discover provided a revised trial design.
3/29/2001	FDA TELEFAX	Comments on prophylaxis superiority trial with Surfaxin or Survanta rescue.
3/19/2001	FDA TELEFAX	Response to alternate proposal
3/26/2001	DSCO Telefax	Follow-up to the 3-22-01 teleconference with the FDA. Provided: a new RDS trial design.
5/21/2001	General Correspondence: Teleconference/Meeting Minutes Protocol Amendment: New Protocol	Protocol KL4-IRDS-06, Discovery's Meeting Minutes from 3/12/01 regarding phase 3 RDS; 3/22/01 regarding phase 3 RDS; 3/29/01 regarding proposed alternative trial; 4/4/01 regarding proposed alternative trial.
5/22/2001	FDA TELEFAX	FDA Meeting Minutes from 4/4/01 regarding proposed alternative trial.
5/22/2001	FDA TELEFAX	FDA Meeting Minutes from 3/29/01 regarding proposed alternative trial.
5/22/2001	FDA TELEFAX	FDA Meeting Minutes from 3/22/01 regarding phase 3 RDS
5/22/2001	FDA TELEFAX	FDA Meeting Minutes from 3/12/01 regarding phase 3 RDS
6/4/2001	General Correspondence	Discovery Hardcopy's of facsimile transmissions: 3/14/ 2001 revised trial design based on agency feedback during the March 12, 2001 meeting, 3/26/01 revised trial design, 3/30/01 revised trial design and 4/4/01 requested a copy of the agency's meeting mins from the 3/12/01 meeting and the telecon held on 3/22/01 & 3/29/01.
8/16/2001	FDA Telefax	Clinical Comments for protocols KL4-IRDS-02 and KL4-IRDS-06

Date	*Type of Document*	*Description*
8/29/2001	Protocol Amendment: New Investigator	Added three (3) new Investigators for protocol KL4-IRDS-02. Attachments: relevant form FDA 1572s and CVs.
8/29/2001	Protocol Amendment: New Investigator	Added one (1) new Investigator for protocol KL4-IRDS-06; Attachments: relevant form FDA 1572 and CV.
9/5/2001	DSCO Telefax	Submitted IND Safety Report - 7 Day Alert (Initial).
9/5/2001	IND Safety Report: 7-Day Alert (Initial)	Submitted IND Safety Report - 7 Day Alert (Initial).
9/12/2001	DSCO Telefax	Submitted IND Safety Report - 7-Day Alert (Initial).
9/13/2001	DSCO Telefax	Submitted IND Safety Report - 7-Day Alert (Initial).
9/13/2001	IND Safety Report: 7-Day Alert (Initial)	Submitted IND Safety Report - 7-Day Alert (Initial).
9/18/2001	DSCO Telefax	Submitted IND Safety Report - 7-Day Alert (Initial).
9/18/2001	IND Safety Report: 7-Day Alert (Initial)	Submitted IND Safety Report - 7-Day Alert (Initial).
9/26/2001	FDA Letter	Clinical and Statistical comments from the FDA on Protocol KL4-IRDS-02/06
9/26/2001	FDA Telefax	Clinical Comments from FDA on Protocol # KL4-IRDS-02 and 06
9/26/2001	DSCO Telefax	Submitted IND Safety Report - 7-Day Alert (Initial).
9/27/2001	IND Safety Report: 15-Day Alert (Initial)	Submitted IND Safety Report - 15 Day Alert (Initial).
9/27/2001	IND Safety Report: 15-Day Alert (Initial)	Submitted IND Safety Report - 15-Day Alert (Initial).
10/1/2001	DSCO Telefax	Submitted IND Safety Report - 15-Day Alert (Initial).
10/1/2001	IND Safety Report: 15-Day Alert (Initial)	Submitted IND Safety Report - 15-Day Alert (Initial)..
10/4/2001	IND Safety Report: 7-Day Alert (Initial)	Submitted IND Safety Report - 7-Day Alert (Initial).
10/19/2001	Protocol Amendment: Change in Protocol	Change Document for Protocol KL4-IRDS-06 Amendment 1; Protocol KL4-IRDS-06 Amendment 1; DSCO's response to Agency's comments regarding KL4-IRDS-06; Agency's comments regarding protocol KL4-IRDS-06.
10/31/2001	DSCO Telefax	Submitted IND Safety Report - 7 Day Alert (Initial).
11/14/2001	IND Safety Report: 7-Day Alert (Initial)	Submitted IND Safety Report - 7 Day Alert (Initial).
11/20/2001	DSCO Telefax	Submitted IND Safety Report - 7 Day Alert.
11/20/2001	DSCO Telefax	Submitted IND Safety Report: 7-Day Alert (Initial).
11/20/2001	Protocol Amendment: New Investigator	Added five (5) new Investigators for Protocol KL4-IRDS-02; Attachments: relevant form FDA 1572s and CVs.
11/20/2001	Protocol Amendment: New Investigator	Added four (4) new Investigators for protocol KL4-IRDS-06; Attachments: relevant form FDA 1572s and CVs.
11/20/2001	IND Safety Report: 7-Day Alert (Initial)	Submitted IND Safety Report - 7 Day Alert.
1/7/2002	Protocol Amendment: Change in Protocol Information Amendment: Clinical	Change document for protocol KL4-IRDS-06 Amendment 2; Protocol KL4-IRDS-06 Amendment 2; Change document for KL4-IRDS-06 Amendment 2 CRF ; CRF for KL4-IRDS-06 Amendment 2.
1/7/2002	Protocol Amendment: New Investigator	Added five (5) new investigators protocol KL4-IRDS-02; Attachments: relevant form FDA 1572s and CVs.
1/20/2002	DSCO Telefax	Submitted IND Safety Report - 7 Day Alert (Initial).

Date	*Type of Document*	*Description*
1/22/2002	Protocol Amendment: Change in Protocol Information Amendment: Clinical	DSCO's response to FDA comments on Protocol KL4-IRDS-02 -US; Agency's comments on Protocol KL4- IRDS-02-US; Change documents for protocol KL4-IRDS-02 -US Amendment 1; Protocol KL4-IRDS-02 -US Amendment 1; Change documents for Protocol KL4-IRDS-02 –US Amendment 1 CRF; Protocol KL4-IRDS-02 -US Amendment 1 CRF; Change document for ICF; Sample ICF.
1/22/2002	Information Amendment: Clinical	Changed document for sample informed consent, and revised sample consent for protocol KL4-IRDS-06.
1/24/2002	DSCO Telefax	Submitted IND Safety Report - 7 Day Alert (Initial).
1/24/2002	IND Safety Report: 7-Day Alert (Initial)	Submitted IND Safety Report - 7 Day Alert (Initial).
1/25/2002	DSCO Telefax	Submitted IND Safety Report - 15 Day Alert (Initial).
1/25/2002	IND Safety Report: 15-Day Alert (Initial)	Submitted IND Safety Report - 15 Day Alert (Initial).
2/7/2002	DSCO Telefax	Submitted IND Safety Report - 7 Day Alert (Initial).
2/18/2002	DSCO Telefax	Submitted IND Safety Report - 7 Day Alert (Initial).
2/18/2002	IND Safety Report: 7-Day Alert (Initial)	Submitted IND Safety Report - 7 Day Alert (Initial).
2/26/2002	IND Safety Report: 7-Day Alert (Initial)	Submitted IND Safety Report - 7 Day Alert (Initial).
2/26/2002	IND Safety Report: 7-Day Alert (Initial)	Submitted IND Safety Report - 7 Day Alert (Initial).
2/27/2002	DSCO Telefax	List of DSCO personnel who will be attending the 3/4/02 teleconference.
2/27/2002	Annual Report	2001 Annual Safety Report covering the period from 8/1/2000 to 7/31/2001.
3/5/2002	Information Amendment: Clinical	Clinical Trial Data and Safety Monitoring Board Standard Operating Procedure Manual.
3/6/2002	FDA Fax	FDA Statistical and clinical comments for submission 103 dated 1/7/02
3/6/2002	Information Amendment: Clinical	Deleted three (3) principal investigators from protocol KL4- IRDS-02
3/19/2002	DSCO Telefax	Submitted IND Safety Report - 7 Day Alert (Initial).
3/22/2002	FDA Fax	Request for info.
4/11/2002	IND Safety Report: 7-Day Alert (Initial) Information Amendment: Clinical	Submitted IND Safety Report - 7 Day Alert (Initial).
4/12/2002	DSCO Telefax	Submitted IND Safety Report - 7 Day Alert (Initial).
4/17/2002	DSCO Telefax	Submitted IND Safety Report - 7 Day Alert (Initial).
4/24/2002	General Correspondence: Response to FDA Request for Information	Response to Request for Information for the sites, includes documents: 3/22/02 FDA faxed information request; and DSCO response.
4/28/2002	DSCO Telefax	Submitted IND Safety Report - 7 Day Alert (Initial).
4/29/2002	General Correspondence: Meeting Minutes General Correspondence: Response to FDA	DSCO's meeting minutes from 3/4/02 teleconference; Agency's comments (3/6/02); DSCO's response to comments; Final Statistical Analysis Plan for IRDS-06, and a draft SOP manual for IRDS-06 adjudication committee.
4/29/2002	IND Safety Report: 7-Day Alert (Initial) Information Amendment: Clinical	Submitted IND Safety Report - 7 Day Alert (Initial). Amendment to the IB in the form of an Investigator Letter.

Date	*Type of Document*	*Description*
4/29/2002	IND Safety Report: 7-Day Alert (Initial) Information Amendment: Clinical	Submitted IND Safety Report - 7 Day Alert (Initial). Amendment to the IB in the form of an Investigator Letter.
4/30/2002	IND Safety Report: 7-Day Alert (Initial) Information Amendment: Clinical	Submitted IND Safety Report - 7 Day Alert (Initial). Amendment to the IB in the form of an Investigator Letter.
5/6/2002	Protocol Amendment: New Investigator Protocol Amendment: Change in Investigator	Added new investigators to protocol KL4-IRDS-02; Attachments: relevant form FDA 1572s and CVs.
5/7/2002	IND Safety Report: 7-Day Alert (Initial) Information Amendment: Clinical	Submitted IND Safety Report - 7 Day Alert (Initial). Amendment to the IB in the form of an Investigator Letter.
5/7/2002	Protocol Amendment: New Investigator	Added new Investigators to protocol KL4-IRDS-02;Attachments: relevant form FDA 1572s and CVs.
5/17/2002	General Correspondence	Correction to Serial No. 123 sent on 5/6/02 which had the incorrect protocol title. This submission contains the correct protocol title.
5/24/2002	DSCO Fax	Submitted IND Safety Report - 15 Day Alert (Initial).
5/24/2002	IND Safety Report: 15-Day Alert (Initial)	Submitted IND Safety Report - 15 Day Alert (Initial).
5/28/2002	DSCO Telefax	Submitted IND Safety Report - 7 Day Alert (Initial).
6/3/2002	Information Amendment: Clinical	Amendment to the IB in the form of an Investigator Letter dated May 28, 2002; The SAE reporting.
6/4/2002	IND Safety Report: 7-Day Alert (Initial) Information Amendment: Clinical	Submitted IND Safety Report-7 Day Alert (Initial). Amendment to the IB in the form of an Investigator Letter.
6/12/2002	Protocol Amendment: New Investigator Protocol Amendment: Change in Investigators	Added new Investigators. Attachments: relevant form FDA 1572s and CV.
6/13/2002	Protocol Amendment: New Investigator Protocol Amendment: Change in Investigators	Added new Investigators to protocol KL4-IRDS-02; Attachments: relevant form FDA 1572s and CVs.
6/14/2002	DSCO Telefax	Submitted IND Safety Report - 15 Day Alert (Initial).
6/14/2002	IND Safety Report: 15-Day Alert (Initial) Information Amendment: Clinical	Submitted IND Safety Report: 15-Day Alert (Initial).
7/1/2002	Protocol Amendment: New Investigators	Added new investigators to protocol KL4- IRDS-02 study; Attachments: relevant form FDA 1572s and CVs.
7/22/2002	DSCO Fax	Submitted IND Safety Report - 7 Day Alert (Initial).
7/26/2002	IND Safety Report - 7 Day Alert (Initial) Information Amendment: Clinical	Submitted IND Safety Report - 7 Day Alert (Initial). Amendment to the IB in the form of an Investigator Letter dated 22 July 2002.
8/19/2002	DSCO Fax	Submitted IND Safety Report - 7 Day Alert (Initial).
8/27/2002	DSCO Fax	Submitted IND Safety Report - 7 Day Alert (Initial).

Date	*Type of Document*	*Description*
9/5/2002	IND Safety Report - 7 Day Alert (Initial) Information Amendment: Clinical	Submitted IND Safety Report - 7 Day Alert (Initial). Amendment to the IB in the form of an Investigator Letter dated 15 August 2002.
9/5/2002	Protocol Amendment: New Investigators	Added new investigators to protocol KL4- IRDS-06. Attachments: relevant form FDA 1572s and CVs.
9/5/2002	Protocol Amendment: New Investigators	Added new investigators to protocol KL4-IRDS-02. Attachments: relevant form FDA 1572s and CVs.
9/23/2002	DSCO Fax	Submitted IND Safety Report - 7 Day Alert (Initial).
9/30/2002	Protocol Amendment: New Investigators	Added new investigators to protocol KL4-IRDS-06. Attachments: relevant form FDA 1572s and CVs.
10/14/2002	General Correspondence: Response to FDA Request for Information	Submitted CIOMS report for patient 793001 enrolled in protocol KL4-IRDS-06 as requested by FDA project manager, Yu, via telephone on September 26, 2002.
10/15/2002	General Correspondence	Requesting the Agency's approval to submit CIOMS reports for SAEs meeting expedited reporting requirement that occurred in protocols KL4-IRDS-06 and KL4-IRDS-02.
10/16/2002	IND Safety Report - 7 Day Alert (Initial) Information Amendment: Clinical	Submitted IND Safety Report - 7 Day Alert (Initial). Amendment to the IB in the form of an Investigator Letter dated October 8, 2002.
11/6/2002	Protocol Amendment: New Investigators Protocol Amendment: Change in Investigators	Added new investigator to protocol KL4-IRDS-02. Attachments: relevant form FDA 1572 and CV.
11/19/2002	Protocol Amendment: New Investigators Protocol Amendment: Change in Investigators	Added new investigators to protocol the KL4-IRDS-06. Attachments: relevant form FDA 1572s and CVs.
11/26/2002	DSCO Fax	Submitted IND Safety Report - 7 Day Alert (Initial).
11/27/2002	FDA TELEFAX	FDA teleconference meeting minutes from March 4, 2002 regarding status of protocol KL4-IRDS-06 and protocol changes submitted January 7, 2002.
12/20/2002	IND Safety Report: 7-Day Alert (Initial) Information Amendment: Clinical	Submitted IND Safety Report: 7-Day Alert (Initial). Amendment to the IB in the form of an Investigator Letter dated 19 December 2002.
1/9/2003	Protocol Amendment: New Investigator Protocol Amendment: Change in Investigator	Added new investigator to protocol KL4-IRDS-02. Attachments: relevant form FDA 1572 and CV.
1/13/2003	Annual Report	2002 Annual Safety Report- dated January 13, 2003
1/15/2003	DSCO Telefax	Reference is made to January 6, 8, and 10, 2003 telephone discussions regarding mortality in the KL4-IRDS-06 clinical trial.
1/20/2003	Protocol Amendment: New Investigator	Added new investigators to protocol KL4-IRDS-06. Attachments: relevant form FDA 1572s and CVs.
1/24/2003	Protocol Amendment: New Investigator	Added new investigator to protocol KL4-IRDS-02. Attachments: relevant form FDA1572 and CV.
2/20/2003	General Correspondence: Response to FDA Request for Info.	Reference is made to January 6, 8 and 10, 2003 discussions regarding mortality in KL4-IRDS-06. This information was previously sent to the FDA via fax on January 15, 2003. Provided data listing.
2/24/2003	FDA TELEFAX	Comments from April 29, 2002, serial no. 119 KL4-IRDS-06 submission. Clinical comments regarding the DAC SOP, statistical comments regarding the final SAP and the DSMB SOP.

Date	*Type of Document*	*Description*
2/26/2003	DSCO Telefax	Submitted IND Safety Report: 7-Day Alert (Initial).
2/27/2003	DSCO Telefax	Submitted IND Safety Report: 7-Day Alert (Initial).
2/28/2003	IND Safety Report: 7-Day Alert (Follow-up)	Submitted IND Safety Report: 7-Day Alert (Follow-up).
3/6/2003	IND Safety Report: 7-Day Alert (Follow-up)	Submitted IND Safety Report: 7-Day Alert (Initial).
3/12/2003	IND Safety Report: 7-Day Alert (Follow-up) Information Amendment: Clinical	Submitted IND Safety Report: 7-Day Alert (Follow-up). Amendment to the IB in the form of an Investigator Letter dated March 6, 2003.
3/12/2003	Information Amendment: Clinical	Amendment to the IB in the form of an Investigator Letter dated 6 March 2003. Reporting SAE.
3/12/2003	IND Safety Report: 7-Day Alert (Follow-up)	Submitted IND Safety Report: 7-Day Alert (Follow-up).
4/4/2003	FDA Letter	Letter from B. Chowdhury, Div. Director, requesting a plan of how Discovery will investigate the Agency's concern regarding the inactivation of Surfactant .
4/4/2003	FDA TELEFAX	Faxed Letter from B. Chowdhury, Div. Director, requesting a plan of how Discovery will investigate the Agency's concern regarding the inactivation of Surfactant.
4/8/2003	DSCO Telefax	To: Yu. From: Schaber. Regarding follow-up to phone conversation of April 7, 2003. Provided COA's for the following lots: 121231; 21492; 41332; and 51212. Additionally provided key points from previous discussions for the review team's consideration.
4/17/2003	Information Amendment: Clinical	Investigator brochure, version 8, dated April 15, 2003.
4/17/2003	General Correspondence: Response to FDA Request for Info.	March 28, 2003 correspondence with Mr. Arlyn Baumgarten, Director, FDA Chicago District Office regarding the status of Surfaxin® batches currently being used in trials; and April 8, 2003 fax that provided the agency with the COA's for the Surfaxin® batches under evaluation.
4/17/2003	General Correspondence: Request for Meeting	Request for Pre-NDA meeting for Surfaxin® in the prevention of RDS.
4/24/2003	DSCO Telefax	To: Yu. From: Rumage. Provided the April 29, 1998 letter from the United States Adopted Names Council (USAN) as follow-up to April 24, 2003 conversation.
4/24/2003	Information Amendment: Clinical	Final statistical analysis plan and table shells for protocol KL4-IRDS-02, entitled, "A Masked, Multicenter, Randomized, Controlled Trial Comparing the Safety and Effectiveness of Surfaxin® (lucinactant) to Curosurf® (poractant alfa) in the Prevention and Treatment of Respiratory Distress Syndrome (RDS) in Premature Neonates."
	General Correspondence: Response to FDA Request for Info.	The Agency's comments regarding protocol KL4-IRDS-06 - dated 02/24/2003 (fax copy); DSCO's response to the Agency's 02/24/2003 comments regarding protocol KL4-IRDS-06.
4/30/2003	General Correspondence	Briefing document for the Pre-NDA Meeting for Surfaxin® in RDS to be held on June 13, 2003 at the FDA Office.
5/16/2003	DSCO Telefax	Submitted IND Safety Report: 7-Day Alert (Initial).
5/30/2003	DSCO Telefax	Provided a response to the agency's letter dated April 4, 2003, received April 9, 2003 regarding the potential inactivation of surfactants when they come into contact wither rubber and/or rubber lubricant.

Date	*Type of Document*	*Description*
6/1/2003	DSCO Telefax	Submitted IND Safety Report: 7-Day Alert (Initial).
6/2/2003	DSCO Telefax	Follow-up to voice message on June 2, 2003 regarding the termination of enrollment of patients in protocol KL4-IRDS-02. Faxed consisted of a copy of the formal submission, serial no. 163.
6/2/2003	Information Amendment: Clinical	Notification to the FDA of termination of enrollment to protocol KL4-IRDS-02.
6/2/2003	General Correspondence: Response to FDA Request for Information	Agency's request dated 4/4/03; Discovery's response provided via fax 5/30/03, and the findings of E. Herting, G. Stichtenoth, and B. Robertson regarding rubber stoppers found in the Lancent, vol. 261, dated 1/25/03, pages 311-313.
6/3/2003	DSCO Telefax	Submitted IND Safety Report: 7-Day Alert (Initial) and IND Safety Report: 15-Day Alert (Initial).
6/4/2003	DSCO Telefax	Provided proposal for a change in manufacturing facility for Surfaxin® based on a recent FDA warning letter September 28, 2000 and FDA form 483s issued on December 17, 2001, August 30, 2002 and February 6, 2003.
6/4/2003	DSCO Telefax	Submitted IND Safety Report: 7-Day Alert (Initial).
6/6/2003	General Correspondence	Proposal for a change in manufacturing facility for Surfaxin® .
6/6/2003	IND Safety Report: 7-Day Alert (Follow-up) IND Safety Report: 15-Day Alert (Follow-up)	Submitted IND Safety Report: 7-Day Alert (Follow-up) and IND Safety Report: 15-Day Alert (Follow-up).
6/10/2003	Protocol Amendment: New Investigator Protocol Amendment: Change in Investigator	Added new investigators to protocol KL4-IRDS-06; Changes in sub-investigators at 11 sites. Attachments: relevant form FDA 1572s and CVs.
6/23/2003	DSCO Telefax	Submitted IND Safety Report: 7-Day Alert (Follow-up).
7/2/2003	IND Safety Report: 7-Day Alert (Follow-up)	Submitted IND Safety Report: 7-Day Alert (Follow-up).
7/8/2003	DSCO Telefax	Provided proposal for an animal model to address the agency's request for a toxicology study of Surfaxin®.
7/10/2003	Protocol Amendment: New Investigator Protocol Amendment: Change in Investigator	Added new investigators to Protocol KL4-IRDS-06; Attachments: relevant form FDA 1572s and CVs.
7/11/2003	DSCO Telefax	Submitted IND Safety Report: 7-Day Alert (Follow-up).
7/11/2003	FDA TELEFAX	FDA's meeting minutes for the June 13, 2003 pre-NDA meeting for Surfaxin in neonatal RDS.
7/11/2003	IND Safety Report: 7-Day Alert (Follow-up)	Submitted IND Safety Report: 7-Day Alert (Follow-up).
7/15/2003	Information Amendment: Clinical	Curriculum Vitae of a new Medical Monitor for Discovery Laboratories, Inc.
7/15/2003	General Correspondence Information Amendment: Pharmacology/Toxicology	Proposal to use a new animal model and supporting final nonclinical study reports.
7/17/2003	DSCO Telefax	Submitted IND Safety Report: 7-Day Alert (Follow-up).
7/23/2003	DSCO Telefax	Submitted IND Safety Report - 15 Day Alert (Follow-up).
7/23/2003	IND Safety Report: 7-Day Alert (Follow-up) IND Safety Report: 15-Day Alert (Follow-up)	Submitted IND Safety Report: 7-Day Alert (Follow-up) and IND Safety Report: 15-Day Alert (Follow-up).
7/24/2003	DSCO Telefax	Provided proposal to submit the NDA.

Date	*Type of Document*	*Description*
7/31/2003	DSCO Telefax	Submitted IND Safety Report - 15 Day Alert (Follow-up # 2).
7/31/2003	DSCO Telefax	Provided proposal to an animal model for the agency requested toxicology study for Surfaxin®.
7/31/2003	General Correspondence	Proposal to submit the Surfaxin® new drug application (NDA).
8/8/2003	DSCO Telefax	Submitted IND Safety Report: 7-Day Alert (Follow-up)..
8/11/2003	DSCO Telefax	Provided Discovery's proposal for the analysis of the co-primary endpoints for protocol KL4-IRDS-06.
8/12/2003	General Correspondence	Proposal to use an animal model for the animal toxicology study of Surfaxin®.
8/15/2003	Information Amendment: Clinical	Amendment to the IB in the form of an Investigator Letter dated 4 August 2003.
8/15/2003	IND Safety Report: 7-Day Alert (Follow-up) IND Safety Report: 15-Day Alert (Follow-up)	Submitted IND Safety Report: 7-Day Alert (Follow-up) and IND Safety Report: 15-Day Alert (Follow-up).
8/19/2003	General Correspondence	Proposal for analysis of the co-primary endpoints for KL4-IRDS-06; A copy of the agency's meeting minutes from the pre-NDA meeting held on June 13, 2003; and Discovery's meeting minutes from the pre-NDA meeting held on June 13, 2003.
8/22/2003	DSCO Telefax	Submitted IND Safety Report: 7-Day Alert (Initial).
8/27/2003	DSCO Telefax	Submitted IND Safety Report: 7-Day Alert (Follow-up) and IND Safety Report: 15-day (Initial).
8/27/2003	IND Safety Report: 7-Day Alert (Initial)	Submitted IND Safety Report: 7-Day Alert (Initial).
8/28/2003	IND Safety Report: 7-Day Alert (Follow-up)	Submitted IND Safety Report: 7-Day Alert (Follow-up).
9/4/2003	IND Safety Report: 15-Day Alert (Initial)	Submitted IND Safety Report: 15-Day Alert (Initial).
9/8/2003	DSCO Telefax	Submitted IND Safety Report: 7-day Alert (Follow-up) and IND Safety Report: 15-day Alert (Follow Up).
9/12/2003	Protocol Amendment: New Investigator Protocol Amendment: Change in Investigator	Added new investigators to protocol KL4-IRDS-06. Attachments: relevant form FDA 1572s and CVs.
9/19/2003	IND Safety Report: 7-Day Alert (Follow-up) IND Safety Report: 15-Day Alert (Follow-up) Information Amendment: Clinical	Submitted IND Safety Report: 7-Day Alert (Follow-up) and IND Safety Report: 15-Day Alert (Follow-up). Amendment to the IB in the form of an Investigator Letter dated 11September2003.
9/25/2003	DSCO Telefax	Submitted IND Safety Report: 7-Day Alert (Follow-up).
9/25/2003	DSCO Telefax	To: Yu. From: Rumage. Provided a new proposal to use a new animal model.
9/25/2003	General Correspondence	Proposal to use a new animal model for the animal toxicology study of Surfaxin®.
10/1/2003	DSCO Telefax	Submitted IND Safety Report: 7-Day Alert (Follow-up) and IND Safety Report: 15-Day Alert (Follow-up).
10/3/2003	DSCO Telefax	Submitted IND Safety Report: 7-Day Alert (Initial).
10/3/2003	DSCO Telefax	Provided Discovery's proposal for the Surfaxin® drug product stability data to be included in the new drug application (NDA).
10/3/2003	General Correspondence	Proposal for the Provision of Drug Product Stability for the New Drug Application (NDA) Filing for Surfaxin® in Respiratory Distress Syndrome (RDS).
10/13/2003	DSCO Telefax	Submitted IND Safety Report: 15-day Alert (Follow-up).

Date	*Type of Document*	*Description*
10/14/2003	FDA TELEFAX	Faxed comments from the FDA (Christine Yu) to Discovery (Christopher Schaber) in response to our proposals for IND 40,287 (serial nos. 174, 175, and 184) regarding submitting the NDA.
10/17/2003	Annual Report	2003 Annual Safety Report- dated October 17, 2003
10/21/2003	DSCO Telefax	Submitted IND Safety Report: 7-Day Alert (Follow-up).
10/21/2003	IND Safety Report: 7-Day Alert (Follow-up)	Submitted IND Safety Report: 7-Day Alert (Follow-up).
10/21/2003	IND Safety Report: 7-Day Alert (Follow-up) IND Safety Report: 15-Day Alert (Follow-up) Information Amendment: Clinical	Submitted IND Safety Report: 7-Day Alert (Follow-up) and IND Safety Report: 15-Day Alert (Follow-up).
10/21/2003	IND Safety Report: 7-Day Alert (Initial) Information Amendment: Clinical	Submitted IND Safety Report: 7-Day Alert (Initial). Amendment to the IB in the form of an Investigator Letter dated 7 October 2003.
10/30/2003	General Correspondence	Supplemental information for the proposal for the analysis of the co-primary endpoints for protocol KL4-IRDS-06.
10/31/2003	DSCO Telefax	Discovery (C. Schaber) fax to the FDA (C. Yu) - provided supplement to August 19, 2003 Co-primary endpoint proposal along with October 29, 2003 from the Data Monitoring Committee.
11/6/2003	DSCO Telefax	Submitted IND Safety Report: 7-Day Alert (Follow-up) and IND Safety Report: 15-day Alert (Follow up).
11/7/2003	FDA TELEFAX	FDA comments in response to serial no. 185 for IND 40,287.
11/12/2003	Protocol Amendment: Change in Investigator	Change in principal investigator for protocol KL4-IRDS-06;Attachments: relevant form FDA 1572 and CV.
11/12/2003	IND Safety Report: 7-Day Alert (Follow-up) IND Safety Report: 15-Day Alert (Follow-up)	Submitted IND Safety Report: 7-Day Alert (Follow-up) and IND Safety Report: 15-Day Alert (Follow-up).
11/14/2003	FDA TELEFAX	FDA comments in response to serial nos. 178 and 190 for IND 40,287.
11/19/2003	Information Amendment: Clinical	Updated Surfaxin® Investigator Brochure, Version 9, dated November 7, 2003 for the IRDS and MAS indications.
11/20/2003	Protocol Amendment: Change in Protocol Information Amendment: Clinical	FDA's Nov. 14, 2003 facsimile transmission; Change document for Protocol KL4-IRDS-06 Amendment 3; Protocol KL4-IRDS-06 Amendment 3-dated November 19, 2003; DSMB SOP Amendment 1 – dated Nov. 17, 2003; Original DSMB SOP - dated Jan. 31, 2002.
11/21/2003	DSCO Telefax	Submitted IND Safety Report: 7-Day Alert (Follow up).
11/21/2003	Information Amendment: Clinical	Amended Statistical Analysis Plan for protocol KL4-IRDS-06 revised to address changes in the primary and secondary endpoints per the agency's comments from the June 13, 2003 pre-NDA meeting.
11/24/2003	IND Safety Report: 7-Day Alert (Follow-up)	Submitted IND Safety Report: 7-Day Alert (Follow-up).
11/25/2003	Information Amendment: Clinical	Official notification to the FDA to stop patient enrollment into the KL4-IRDS-06 clinical trial based on the discretion of the DSMB due that the target number of events had been reached for the co-primary endpoints for the study.

Date	*Type of Document*	*Description*
11/29/2003	Information Amendment: Clinical	Cover letter dated November 22, 2003 from the DSMB (Joe Massaro. PhD) to Badrul Chowdhury, MD, PhD in which the DSMB provided the revised SAP for protocol KL4-IRDS-06.
12/4/2003	Information Amendment: Clinical	Revised Adjudication SOP manual for Protocol KL4-IRDS-06 Amendment 2- dated November 19, 2003.
1/6/2004	DSCO Telefax	Submitted IND Safety Report: 7 Day Alert (Follow up) and IND Safety Report: 15- DAY (Follow up).
1/7/2004	IND Safety Report: 7-Day Alert (Follow-up) IND Safety Report: 15-Day Alert (Follow-up)	Submitted IND Safety Report: 7-Day Alert (Follow-up) and IND Safety Report: 15-Day Alert (Follow-up).
1/12/2004	DSCO Telefax	To: Yu. From: Rumage. Proposal for RDS NDA. .
1/16/2004	IND Safety Report: 7-Day Alert (Follow-up)	Submitted IND Safety Report: 7-Day Alert (Follow-up).
1/20/2004	DSCO Telefax	Submitted IND Safety Report: 7-Day Alert (Follow-up).
1/31/2004	DSCO Telefax	Submitted IND Safety Report: 7-Day Alert (Follow-up).
2/3/2004	DSCO Telefax	Submitted IND Safety Report: 7-Day Alert (Follow-up).
2/3/2004	IND Safety Report: 7-Day Alert (Follow-up)	Submitted IND Safety Report: 7-Day Alert (Follow-up).
2/11/2004	DSCO Telefax	Submitted IND Safety Report: 7-Day Alert (Follow-up).
2/11/2004	Information Amendment: Clinical	Change document for KL4-IRDS-06 SAP , version 5; KL4-IRDS-06 Statistical Analysis Plan , version 5 (2 volumes).
3/8/2004	DSCO Telefax	Submitted IND Safety Report: 7-Day Alert (Follow-up).
3/8/2004	IND Safety Report: 7-Day Alert (Follow-up)	Submitted IND Safety Report: 7-Day Alert (Follow-up).
3/11/2004	IND Safety Report: 7-Day Alert (Follow-up)	Submitted IND Safety Report: 7-Day Alert (Follow-up).
3/16/2004	Protocol Amendment: Change in Investigator	Changes in principal investigators and sub-investigators for protocol KL4-IRDS-02. Attachments: relevant form FDA 1572s and CVs.
3/22/2004	Protocol Amendment: Change in Investigator	Changes in sub-investigators at 11 sites for Protocol KL4-IRDS-06.
3/23/2004	DSCO Telefax	Submitted IND Safety Report.
3/23/2004	DSCO Telefax	To: Yu. From: Rumage. Questions to the FDA regarding the submission of IRDS NDA # 21,746.
4/5/2004	DSCO Telefax	Submitted IND Safety Report 7-DAY Alert (Follow-up).
4/19/2004	Information Amendment: Chemistry/Microbiology	CMC amendment to allow for a change in manufacturing facility.
4/23/2004	FDA Telefax	To: World Courier, Inc. From: R. Meja (FDA) Provided notice of FDA Action, entry no. 113-2657439-6 regarding the importation of study drugs.
4/30/2004	IND Safety Report: 7-Day Alert (Follow-up)	IND Safety Report: 7-Day Alert (Follow-up).
5/18/2004	DSCO Telefax	To: R. Meja From: K. Tsokas Response to April 23, 2004 notice of FDA Action, entry no. 113-2657439-6 for clinical study drugs.
5/24/2004	FDA Telefax	To: World Courier, Inc. From: R. Meja (FDA) Provided notice of FDA Action to release Exosurf®.
6/15/2004	FDA Telefax	Submitted IND Safety Report: 7-Day Alert (Follow up).
6/16/2004	IND Safety Report: 7-Day Alert (Follow-up)	Submitted IND Safety Report: 7-Day Alert (Follow-up).
6/25/2004	DSCO Telefax	Submitted IND Safety Report: 7-Day Alert (Follow-up).

Date	*Type of Document*	*Description*
6/29/2004	IND Safety Report: 7-Day Alert (Follow-up)	Submitted IND Safety Report: 7-Day Alert (Follow-up).
7/2/2004	DSCO Telefax	Submitted IND Safety Report: 7-Day Alert (Follow-up).
7/6/2004	IND Safety Report: 7-Day Alert (Follow-up)	Submitted IND Safety Report: 7-Day Alert (Follow-up).
7/28/2004	DSCO Telefax	Submitted IND Safety Report: 7- Day Alert (Follow-up).
7/29/2004	Information Amendment: Clinical	Text portion of the KL4-IRD-02 clinical study report dated March 15, 2004 along with appendix 16.1.9 (Statistical Analysis Plan). (2 volumes).
7/30/2004	Information Amendment: Clinical	Text portion of the KL4-IRD-06 clinical study report dated March 21, 2004 along with appendix 16.1.9 (Statistical Analysis Plan). (2 volumes).
8/4/2004	IND Safety Report: 7-Day Alert (Follow-up)	Submitted IND Safety Report: 7-Day Alert (Follow-up).
8/26/2004	General Correspondence	Proposal for a 3b clinical protocol for the treatment of premature infants with RDS; Request for Teleconference if necessary.
10/6/2004	Information Amendment: Clinical	Curriculum Vitae of new Medical Monitor for IND 40,287.
10/22/2004	Protocol Amendment: New Protocol	New Protocol, KL4-BPD-01 entitled "A Randomized, Double-blind, Placebo-Controlled Trial to Assess the Safety and Efficacy of Surfaxin® (lucinactant), in Very Low Birth Weight (VLBW) Infants at Risk for Developing Bronchopulmonary Dysplasia".
11/17/2004	DSCO Telefax	Submitted IND Safety Report: 7-Day Alert (Follow up) and IND Safety Report: 15-Day Alert (Follow up).
11/17/2004	General Correspondence	Notification of change of address, telephone number, and fax number for Discovery's corporate office.
11/18/2004	Annual Report	2004 Annual Safety Report covering the period August 1, 2003 through July 31, 2004 (2 volumes).
11/24/2004	FDA Telefax FDA Minutes	Agency's meeting minutes from October 27, 2004 teleconference regarding clarification on issues arising from two submissions to IND 40,287 and NDA 21-746.
11/30/2004	IND Safety Report: 7-Day Alert (Follow-up) IND Safety Report: 15-Day Alert (Follow-up)	Submitted IND Safety Report: 7-Day Alert (Follow-up) and IND Safety Report: 15-Day Alert (Follow-up).
12/29/2004	Information Amendment: Pharmacology/Toxicology	Notification of the transfer of documents/specimens.
1/11/2005	Information Amendment: Pharmacology/Toxicology	Amendment final study reports.
1/14/2005	Information Amendment: Clinical	Submitted CIOMS reports for the following patients enrolled in KL4-IRDS-02: 201002, 211001, 211002, 212002, 221002, 302006, 702005, 711002, 731003, and 812003.
2/2/2005	DSCO Telefax	Submitted IND Safety Report 15-Day Alert: (Follow up).
2/2/2005	IND Safety Report: 15-Day Alert (Follow-up)	Submitted IND Safety Report: 15-Day Alert (Follow-up).
3/11/2005	Information Amendment: Clinical	Change in medical monitor.
4/19/2005	Information Amendment: Clinical	Provided CIOMS reports for the following patients enrolled in KL4-IRDS-06 who experienced SAEs that met expedited reporting criteria: 012003, 043007, 062002, 063001, 063005, 082001, 201002, 553003, 701004, 731001, 732002, 751012, and 782002.

Date	*Type of Document*	*Description*
5/4/2005	Protocol Amendment: Change in Investigators Protocol Amendment: Deleted Investigators Information Amendment: Clinical	Final change in investigators for protocols KL4-IRDS-02 and KL4-IRDS-06.
8/26/2005	Information Amendment: Clinical	Text portion of the Amended short term and long term Clinical Study Reports for KL4-IRDS-02 and KL4-IRDS-06; KL4-IRDS-02 Volume 1 of 2; KL4-IRDS-06 Volume 2 of 2.
1/24/2006	Annual Report	2005 Annual Report covering the period August 1, 2004 through July 31, 2005.
12/18/2006	Annual Report	2006 Annual Report covering the period August 1, 2005 through September 4, 2006.
3/20/2007	Information Amendment: Chemistry, Manufacturing, and Controls	CMC Amendment in CTD format to support clinical trials using 30 mg/mL drug product. (3 Volumes)
5/11/2007	Information Amendment: Pharmacology/Toxicology	Meeting Minutes from the 21DEC2006 Type C meeting for NDA 21-746; Discovery's proposal for the conduct of a SURFAXIN impurity qualification study in ferrets; Literature references
6/26/2007	FDA Telefax	FDA comments regarding pharmacology/toxicology proposal submitted May 11, 2007 (Serial No. 295).
1/4/2008	Annual Report	2007 Annual Report covering September 5, 2006 - September 4, 2007. IB Edition 11 (December 11, 2007) included as an attachment.
1/4/2008	Information Amendment: Pharmacology/Toxicology	Two Nonclinical Study Reports: Bolus Delivery of Lucinactant in Ventilated Preterm Lambs and Acute Intra-Tracheal Instillation Toxicity Study of Lucinactant Impurities.
10/30/2008	Annual Report	2008 Annual Report covering the period September 5, 2007 through September 4, 2008
11/21/2008	Information Amendment: Pharmacology/Toxicology	Preclinical study entitled "Effect of Intratracheal Lucinactant on Respiratory System Compliance in Ventilated Very Preterm Lambs"

Exhibit I

SURFAXIN® NDA 21-746 Brief Description of Significant Events

Date	*Type of Submission*	*Description of Submission*
4/13/2004	Original NDA Application	Discovery Labs filed the original NDA application for SURFAXIN.
4/14/2004	Original NDA Application - Field Copy	Provided the field copy to the North Brunswick Field Office.
4/26/2004	DSCO Facsimile Response to FDA Request for Additional Information	Provided via facsimile the October 18, 1995 letter granting the Scripps Research Institute Orphan Drug Designation for KL_4 Surfactant (currently known as SURFAXIN) to be included in Module 1, Section 1.5 of NDA 21-746 as follow-up to the April 26, 2004 phone conversation with C. Yu (FDA).
4/27/2004	General Correspondence Response to FDA Request for Additional Information	Provided the October 18, 1995 letter granting the Scripps Research Institute Orphan Drug Designation for KL_4 Surfactant (currently known as SURFAXIN) to be included in Module 1, Section 1.5 of NDA 21-746 as follow-up to the April 26, 2004 facsimile.
4/28/2004	General Correspondence Response to FDA Request for Additional Information	Provided the North Brunswick Field Office with the October 18, 1995 letter granting the Scripps Research Institute Orphan Drug Designation for KL_4 Surfactant (currently known as SURFAXIN) to be included in Module 1, Section 1.5 of NDA 21-746.
5/17/2004	General Correspondence Response to FDA Request for Additional Information	Provided response to the May 14, 2004 request for information to the Division of Scientific Investigations. Documents for KL4-IRDS-06 submitted were: Protocol & amends; Blank case report form; Sample ICF; Description of primary endpoints; Table 1.A; Table 1.B; Table 2.A; Table 2.B. (2 volumes).
5/17/2004	FDA Correspondence	Original receipt for the NDA application for NDA 21-746 (received date 05/21/2004). Also, facsimile receipt for the NDA application for NDA 21-746 (received date 05/18/2004).
5/24/2004	General Correspondence Response to FDA Request for Additional Information DSCO Facsimile	Provided response to the May 21, 2004 request for information to the Division of Scientific Investigations.
6/11/2004	DSCO Facsimile	Provided contact Information for the principal investigators at sites 8, 30, 72 and 75 as requested by the FDA.
6/15/2004	General Correspondence Response to FDA Request for Additional Information	Provided response to June 4, 2004 request for five desk copies of Module 1, and the original NDA cover letter and Form FDA 356h on CD-ROM in PDF.
6/15/2004	General Correspondence Response to FDA Request for Additional Information	Provided requested information from June 9, 2004 to the Division of Scientific Investigations.
6/16/2004	DSCO Facsimile	Copy of the cover letter regarding: Requested information from June 9, 2004 to the Division of Scientific Investigations was forwarded via facsimile to C. Yu (FDA)
6/22/2004	DSCO Correspondence Response to FDA Request for Additional Information	Provided the Philadelphia District Office with a CD-ROM of the CMC portion (Module 3) of NDA 21-746-dated 4/13/2004.
6/25/2004	FDA Correspondence	Original 74-day letter from the Division. Acceptance of NDA 21-746 (June 12, 2004) received date 07/01/2004. Faxed copy of Original 74-day letter from the Division(received date 06/28/2004).
6/29/2004	DSCO Correspondence	Provided requested information from June 23, 2004 regarding CMC information.

Date	*Type of Submission*	*Description of Submission*
7/1/2004	DSCO Correspondence	Provided request for the division's acceptance of proposed submission of clinical information.
7/15/2004	FDA Facsimile FDA Request for Additional Information	Request for clinical information for patients enrolled in protocol KL4-IRDS-06 in a tabular format preferably for all three study drugs, Exosurf®, Survanta®, and SURFAXIN®.
7/20/2004	DSCO Facsimile FDA Request for Additional Information	Provided a template of the listings of SAEs through 36 weeks PCA, day of withdrawal of consent, deaths, date of onset, SOC/PT, and listing of primary endpoint related data from CRF and Adjudication results for sites 08, 30, 72, & 75.
7/29/2004	General Correspondence Response to FDA Request for Additional Information	Provided two copies of the following information: primary endpoint related data; treatment assignment; and SAEs through 36 weeks PCA, day of withdrawal of consent, or death for sites 08, 30, 72, and 75.
8/10/2004	DSCO Correspondence FDA Request for Additional Information	Provided R. Rodriguez (FDA -Puerto Rico) with a copy of Module 3 on CD-ROM. Additionally, provided a copy of the cover letter that was sent to R. Rodriguez to C. Yu (FDA) and L. Adams (DFI) via facsimile.
8/16/2004	Form FDA 483	Form FDA 483 for the inspection that occurred from August 10, 2004 - August 16, 2004.
8/16/2004	General Correspondence Response to FDA Request for Additional Information	Provided a response to the agency's July 15, 2004 request for additional information which includes a tabular summary of requested endpoints by batch for protocol KL4-IRDS-06.
8/18/2004	FDA Letter	Letter from the Philadelphia District office regarding Discovery's Doylestown's facility.
8/24/2004	DSCO Facsimile Response to FDA Request for Additional Information	Provided contact information for inspection at the Doylestown office via facsimile.
9/3/2004	General Correspondence Response to FDA Request for Additional Information	Provided a response to DSI's August 25, 2004 request for additional clinical information: patient ID, birth date and time, gestational age, birth weight, and date and time of dose for sites 08, 30, 72, and 75.
9/13/2004	General Correspondence Response to Form FDA 483	Provided a response to the Form FDA 483 issued on August 16, 2004 to both the Philadelphia District Office (T. Gardine) and the Maryland Office (B. Chowdhury).
9/14/2004	FDA Letter	Original receipt from the Philadelphia District Office for Discovery's September 13, 2004 response to the Form FDA 483 issued on August 16, 2004.
9/24/2004	E-Mail Correspondence	The FDA requested additional information regarding the DSMB's final SOP and requested the interim statistical analyses reports for the co-primary endpoints for the NDA.
9/28/2004	DSCO Facsimile Response to FDA Request for Additional Information	Provided with the original KL4-IRDS-06 Adjudication Committee Manual SOP and four representative ballots utilized by the Adjudication Committee via facsimile.
9/30/2004	DSCO Facsimile Response to FDA Request for Additional Information	Provided a sample adjudication data tracking form and a sample data listing that identifies with Adjudication Committee members voted on each patient.
9/30/2004	General Correspondence	Provided Safety Update and Clinical Update.
10/4/2004	DSCO Facsimile Response to FDA Request for Additional Information	Provided FDA LA District Office with a copy of analytical test method, DP-018.
10/4/2004	DSCO Facsimile Response to FDA Request for Additional Information	Provided a data listing which provides the details the patients evaluated by each adjudicator as part of the process of adjudicating the endpoints for the KL4-IRDS-06 study.
10/7/2004	DSCO Facsimile	Provided an updated list of patients evaluated by each

Date	*Type of Submission*	*Description of Submission*
	Response to FDA Request for Additional Information	adjudicator and a change document for the Adjudication Committee manual SOP.
10/8/2004	FDA Letter Response to FDA Request for Additional Information	Provided a response to the September 24, 2004 e-mail regarding the DSMB SOP and reports detailing the conduct and results of any interim analyses of the co-primary endpoints of the KL4-IRDS-06 study.
10/19/2004	General Correspondence/CMC Amendment	Provided DSCO's response to the Division's 74 Letter dated June 25, 2004.
10/21/2004	FDA Letter	Form FDA 483 issued for inspection held on October 13, 15, 18, and 21, 2004.
10/22/2004	DSCO Facsimile Response to FDA Request for Additional Information	Provided a list of hotel accommodations and transportation services for the upcoming inspections. (Note: Date on fax transmittal form is incorrectly recorded as 10/22/2003 s/b 10/22/2004)
10/28/2004	DSCO Facsimile Response to FDA Request for Additional Information	Provided certifications for the four sites who are going to be audited (08, 30, 72, and 75) stating they are available on the scheduled dates of inspection.
11/1/2004	General Correspondence Response to FDA Request for Additional Information DSCO Facsimile	Provided relevant information for the DSMB in response to the agency request from the October 27, 2004 teleconference. (3 Volumes)
11/3/2004	General Correspondence Response to FDA Request for Additional Information DSCO Facsimile	Provided two replacement pages to the November 1, 2004 DSMB submission. A copy of the cover letter was also provided via facsimile to C. Yu (FDA).
11/15/2004	General Correspondence Response to Form FDA 483	Provided additional information for the response to the Form FDA 483 issued on August 16, 2004 to both the Philadelphia District Office and the Division. Initial response was provided September 13, 2004.
11/17/2004	General Correspondence	Notification of Change of Corporate Address to 2600 Kelly Road, Warrington, PA 18976
11/22/2004	FDA Facsimile FDA Request for Additional Information	Request for additional information for the patients for the KL4-IRDS-06 study.
11/23/2004	General Correspondence Response to Form FDA 483	Provided a response to the Form FDA 483 issued on November 2, 2004. Copy provided to the Division and the LA District Office.
11/24/2004	FDA Meeting Minutes	Agency's meeting minutes from October 27, 2004 teleconference.
11/24/2004	DSCO Facsimile Response to FDA 483	Provided November 23, 2004 cover letter in response the FDA 483 issued on November 2, 2004.
11/24/2004	General Correspondence FDA Facsimile Response to FDA 483	Provided a response regarding additional information requested during the inspection held at Discovery from Nov. 8-10, 2004.
11/29/2004	General Correspondence Response to FDA Request for Additional Information	Provided a complete copy of the submission of November 24, 2004 which included a description of actions taken at three adjudication meetings, a list of patients adjudicated at the corresponding meetings, and copies of 17 requested patient files. (2 Volumes).
12/1/2004	Response to FDA Request for Additional Information	Provided a response the FDA's November 22, 2004 facsimile regarding additional information for patients who died by day 14 regarding relationship to RDS.
12/1/2004	Response to FDA Request for Additional Information Response to Form FDA 483	Provided a complete copy of the November 15, 2004 submission.

Date	*Type of Submission*	*Description of Submission*
12/1/2004	DSCO Facsimile	Provided a copy of the November 24, 2004 letter to DSI in response to additional items requested during the agency's visit November 8-10, 2004.
12/8/2004	General Correspondence Clarification to NDA Submission Dated April 13, 2004	Provided a list the SAS decodes/codes of countries in KL4-IRDS-02 and KL4-IRDS-06.
12/8/2004	General Correspondence Clarification to NDA Submission Dated December 1, 2004	Provided the electronic document room with a copy of the SAS Data Transport File provided on December 1, 2004. CD-ROM also included cover letters, 356h form, and corresponding SAS decodes/codes.
12/8/2004	General Correspondence Clarification to NDA Submission Dated December 1, 2004	Provided a list the SAS decodes/codes of the adjudicators and relationship of death to RDS.
12/9/2004	DSCO Facsimile	Provided a copy of the 3 cover letters submitted on December 8, 2004 in response to the December 7, 2004 telephone request.
12/14/2004	FDA Facsimile FDA Request for Additional Information	Request for additional information regarding the process of the adjudication committee's votes.
12/17/2004	General Correspondence DSCO Facsimile Clarification to NDA Submission Dated December 1, 2004	Provided clarification for the occurrence of a committee vote when 2 adjudicators agreed and clarification for two contradictory votes from the same adjudicator. Copy of cover letters also sent via facsimile to C. Yu (FDA).
12/20/2004	General Correspondence Response to FDA Request for Additional Information	Provided Dr. Chowdhury a letter stating that new CD-ROMS were provided to the EDR.
12/20/2004	General Correspondence Response to FDA Request for Additional Information	Provided new CD-ROMS to the EDR as clarification to 11/29/04 submission
12/21/2004	DSCO Facsimile	Provided copies of submissions sent on 12/20/04 to the EDR and Dr. Chowdhury.
12/21/2004	Form FDA 483	Form FDA 483 for Dec. 20 - Dec. 21, 2004 inspection at Doylestown facility.
12/28/2004	General Correspondence Response to FDA Request for Additional Information	Provided the Electronic Document Room (EDR) with a copy of the Draft Package Insert in Word. Additionally, provided the Division with a copy of the cover letter sent to the EDR.
12/29/2004	General Correspondence	Provided notification to the agency of the transfer of specimens and documentation from Provident Preclinical, Inc. to Discovery and EPL Archives.
12/29/2004	DSCO Facsimile Response to FDA Request for Additional Information	Provided copies of the cover letters submitted to the EDR and Pulmonary Division on December 28, 2004.
1/4/2005	General Correspondence Clarification to Original NDA Submission Dated April 13, 2004 DSCO Facsimile	Provided notification to the agency that DMF No. 17159 was updated as requested on Dec. 9, 2004. Also provided a copy of the cover letter via fax.
1/6/2005	DSCO Facsimile	Provided a copy of the cover letter for Discovery's January 4, 2005 submission to NDA 21-746 regarding the amended DMF No. 17159
1/6/2005	FDA Facsimile	Request for clarification regarding the differences of abnormal findings in protocols KL4-IRDS-02 and KL4-IRDS-06; additionally, whether or not ultrasounds were used.
1/6/2005	General Correspondence Response to Form FDA 483	Provided a response to the Form FDA 483 issued on Dec. 21, 2004 to both the Philadelphia District Office and the Pulmonary Division.
1/7/2005	DSCO Facsimile	Provided copies of the cover letters provided to both the

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		Philadelphia District Office and the Pulmonary Division in response to the Form FDA 483 issued on Dec. 21, 2004.
1/7/2005	FDA Letter Establishment Inspection Report	Provided a copy of the establishment inspection report for the inspection conducted at UCSD from November 1 - November 2, 2004.
1/10/2005	General Correspondence Response to FDA Request for Additional Information DSCO Facsimile	Discovery's response to the agency's Jan. 6, 2005 facsimile and Jan. 10, 2005 telephone conversation regarding the differences of abnormal findings in protocols KL4-IRDS-02 and KL4-IRDS-06; additionally, whether or not ultrasounds were used. Also provided a copy of the submission via facsimile. (copy of the agency's Jan. 6, 2005 facsimile included).
1/12/2005	General Correspondence Response to Form FDA 483	Provided a response to the Form FDA 483 issued on Dec. 9, 2004 to both the Philadelphia District Office and the Pulmonary Division.
1/12/2005	DSCO Facsimile	Resent Discovery Jan. 10, 2005 fax which included Discovery's response to the comments received from the agency on Jan. 6, 2005 regarding the differences of abnormal findings in protocols KL4-IRDS-02 and KL4-IRDS-06; additionally, whether or not ultrasounds were used.
1/13/2005	FDA Letter General Correspondence	Acknowledgement of receipt of Discovery's January 12, 2005 submission to the Philadelphia District Office regarding DSCO responses to the FDA Form 483
1/14/2005	FDA Facsimile FDA Meeting Minutes	FDA Meeting Minutes from January 10, 2005 Teleconference.
1/31/2005	General Correspondence Response to FDA 483 Laureate Letter	Provided a response to the Form 483 issued on January 21, 2005 to the Parsippany, NJ Field Office.
1/31/2005	General Correspondence Response to Form FDA 483 DSCO Facsimile	Provided a copy of response to the Form 483 issued on January 21, 2005 to the Pulmonary Division. Additionally, provided a copy of cover letters along with attachments 1 and 2 via facsimile.
2/3/2005	FDA Letter Establishment Inspection Report	Provided a copy of the establishment inspection report for the inspections conducted August 10 - 16, and December 20 - 21, 2004 at Discovery's Doylestown facility.
2/11/2005	General Correspondence	Response to FDA meeting minutes from January 10, 2005 teleconference.
2/11/2005	FDA Facsimile	Provided approvable letter for NDA 21-746 which includes deficiencies noted during the review process.
2/11/2005	FDA Letter	Provided approvable letter for NDA 21-746 which includes deficiencies noted during the review process.
2/17/2005	General Correspondence	Notification of intent to file an amendment to NDA 21-746 in response to the February 11, 2005 approvable letter.
2/17/2005	DSCO Facsimile	Notification of intent to file an amendment to NDA 21-746 in response to the February 11, 2005 approvable letter.
3/18/2005	General Correspondence FDA Facsimile	Request for clarification to the approvable letter dated February 11, 2005 from the agency. Cover letter also sent via facsimile.
3/20/2005	General Correspondence FDA Letter	Comments from the FDA regarding the SOP Manual for the KL4-IRDS-06 Adjudication Committee (Nov. 19, 2003) and request for corrections.
3/31/2005	General Correspondence	Update to manufacturing activities.
4/1/2005	General Correspondence	Provided Discovery's clarification and proposal to the Agency's February 11, 2005 approvable letter regarding the CMC section of NDA 21-746.
4/2/2005	DSCO Facsimile	Provided a copy of the cover letter and Discovery's clarification

Date	*Type of Submission*	*Description of Submission*
		and proposal to the Agency's February 11, 2005 approvable letter regarding the CMC section of NDA 21-746.
4/8/2005	General Correspondence DSCO Facsimile DSCO E-mail	Request for the division's acceptance of proposed submission of clinical information for the safety update. Cover letter sent via e-mail and facsimile.
5/11/2005	FDA Letter	FDA's response to submission dated 04/08/2005- submission of clinical information for safety update
6/8/2005	General Correspondence DSCO Facsimile	Request for a teleconference to discuss April 1, 2005 CMC points of clarification document sent via courier and fax.
6/17/2005	General Correspondence FDA Letter	Notification of date and time of CMC points of clarification teleconference: July 29, 2005 (11:00 AM - 12:00 PM), a tentative list of attendees. 12 copies of meeting package requested.
7/6/2005	General Correspondence	Provided copies of the July 29, 2005 Meeting Package regarding the CMC section of NDA 21-746.
7/13/2005	General Correspondence Response to FDA Request for Additional Information	Provided a copy of response to the remaining items noted on the Form 483 dated January 21, 2005.
7/27/2005	General Correspondence DSCO Facsimile	Discovery's request to cancel the July 29, 2005 teleconference to discuss the CMC section of NDA 21-746 sent via FedEx and fax.
7/29/2005	PDUFA Response	Provided the North Brunswick Field Office with a copy of Volumes 1, 153-157.
7/29/2005	PDUFA Response	Provided a response to the February 11, 2005 PDUFA letter (157 Volumes) to the Pulmonary Division. Copy of cover letters sent to C. Yu via facsimile.
8/16/2005	FDA Facsimile	FDA Comments regarding Discovery's July 29, 2005 PDUFA response.
10/5/2005	General Correspondence DSCO Facsimile	Provided the complete PDUFA response in response the approvable letter dated February 11, 2005 and the August 16, 2005 facsimile to the Agency and the field office.
10/20/2005	FDA Facsimile FDA Letter	Acknowledgment of the October 5, 2005 PDUFA response, which is considered a class 2 response to the February 11, 2005 approvable letter. The user fee goal date is April 6, 2006.
10/24/2005	General Correspondence	DSCO response to the FDA request for additional copies of 10/05/05 Resubmission- Complete Response to NDA Approval Letter-dated 02/11/05 and 08/16/05 FDA Fax.
11/2/2005	General Correspondence	DSCO response to the FDA request for additional information.
11/22/2005	FDA Facsimile	Request for annotated amended clinical study reports for KL4-IRDS-02 and KL4-IRDS-06 as well as the ISE, or written guides specifying the changes in the clinical study reports and ISE submitted in the PDUFA response.
11/23/2005	General Correspondence DSCO Facsimile	Provided a type C meeting request to gain agreement with the Agency regarding the appropriateness of data submitted in Discovery's October 5, 2005 PDUFA response in relation to drug product impurity qualification and analytical methodology and confirm there is no additional issues pending with the NDA.
12/2/2005	FDA Facsimile FDA Letter	Notification that our November 23, 2005 meeting request would not be considered productive at this time. (electronic version- date received 12/03/2005 and fax version- date received 12/02/2005).
12/9/2005	DSCO Facsimile General Correspondence Response to FDA Request for	Provided requested clinical information from the FDA's November 23, 2005 fax regarding the KL4-IRDS-02 and KL4-IRDS-06 clinical study reports and the SURFAXIN Integrated

Date	*Type of Submission*	*Description of Submission*
	Additional Information	Summary of Efficacy. (3 Volumes)
1/4/2006	DSCO Facsimile General Correspondence Response to FDA Request for Additional Information	Provided notification of transfer of ownership of manufacturing facility.
1/20/2006	DSCO Facsimile General Correspondence	Provided additional information regarding the transfer of ownership of the SURFAXIN manufacturing facility .
1/20/2006	DSCO Letter General Correspondence	Provided additional information regarding the transfer of ownership of the SURFAXIN manufacturing facility.
2/7/2006	DSCO Letter General Correspondence	Provided "Coming Soon" promotional pieces (advertisement and CD-ROM) for SURFAXIN to DDMAC and the Pulmonary Division.
3/2/2006	DSCO Letter General Correspondence	Provided updated draft vial and carton labels for SURFAXIN in response to the February 11, 2005 approvable letter.
3/31/2006	FDA Letter General Correspondence	Provided approvable letter for NDA 21-746 which includes deficiencies noted during the second review process.
3/31/2006	FDA Facsimile General Correspondence	Provided approvable letter for NDA 21-746 which includes deficiencies noted during the second review process.
4/6/2006	DSCO Letter General Correspondence	Notification of Intent to File an Amendment to the Approvable Letter Dated March 31, 2006.
5/23/2006	General Correspondence Response to Form FDA 483	Provided a copy of the May 19th submission to the North Brunswick, NJ District Office to the Form FDA 483 issued on April 7, 2006 for Discovery, Totowa to the Division. (copy of Form 483 included).
5/31/2006	General Correspondence Response to Form FDA 483	Provided a copy of the May 19th submission sent to the North Brunswick, NJ District Office in response to the Form FDA 483 issued on April 7, 2006 for Discovery, Totowa to the Parsippany Office.
9/1/2006	FDA Letter Establishment Inspection Report	Provided a copy of the Establishment Inspection Report (EIR) for the inspection conducted March 23, 2006 - April 7, 2006 at Discovery's Totowa facility.
9/27/2006	General Correspondence Meeting Request/Meeting Package	Request for a Type C meeting with the FDA to discuss CMC deficiencies identified in the March 31, 2006 Approvable Letter. Submission also serves as meeting package.
9/27/2006	DSCO Facsimile Meeting Request/Meeting Package	Provided a copy of the Type C meeting request/package for NDA 21-746 without attachments via facsimile.
10/6/2006	General Correspondence	Provided the FDA with Desk Copies of Type C Meeting Request/Meeting Package.
10/9/2006	General Correspondence	Provided the FDA with additional Desk Copies of Type C Meeting Request/Meeting Package.
10/16/2006	FDA Facsimile	Confirmation of December 21, 2006 Type C Meeting at 3:00 PM to discuss deficiencies noted in the FDA's March 31, 2006 letter.
11/16/2006	DSCO Facsimile	Provided supplement to September 27, 2006 meeting package (excluding two appendices) via fax which includes information regarding the qualification of the SURFAXIN drug product and drug substance impurities.
11/16/2006	General Correspondence Supplement to September 27, 2006 Meeting Package	Provided supplement to September 27, 2006 meeting package which includes information regarding the qualification of the SURFAXIN drug product and drug substance impurities.
11/27/2006	DSCO Facsimile Supplement to September 27, 2006 Meeting Package	Provided second supplement to September 27, 2006 meeting package via fax regarding the in-vivo bioassay and the production of new process validation batches.

Date	*Type of Submission*	*Description of Submission*
11/27/2006	General Correspondence Supplement to September 27, 2006 Meeting Package	Provided second supplement to September 27, 2006 meeting package regarding the in-vivo bioassay and the production of new process validation batches.
12/14/2006	DSCO Facsimile General Correspondence	Provided revised list of meeting attendees for the December 21, 2006 meeting.
12/20/2006	FDA Facsimile	Comments for December 21, 2006 Meeting regarding questions contained in Discovery's submissions dated September 27, November 16 and November 27, 2006.
1/8/2007	General Correspondence Meeting Minutes	Provided a copy of Discovery's meeting minutes from the December 21, 2006 Meeting.
1/8/2007	DSCO Facsimile Meeting Minutes	Provided a copy of Discovery's meeting minutes from the December 21, 2006 Meeting via fax (Attachment not included in fax).
1/18/2007	FDA Facsimile Meeting Minutes	Agency's meeting minutes from the December 21, 2006 Meeting.
9/10/2007	General Correspondence Safety Updates	Request for FDA Feedback on Discovery's Proposed Extent and Format of the Safety Update Report.
10/22/2007	FDA Letter FDA Request for Additional Information	Agency's comments on Discovery's request sent 9/10/07 for the proposed extend and format of the requested safety update report (electronic version received 10/22/2007 and US mail version received 10/25/2007).
10/31/2007	Resubmission of NDA Application	Provided Discovery's complete response to the Agency's March 31, 2006 Approvable Letter (3 Sets of 16 volumes).
11/2/2007	Resubmission of NDA Application - Field Copy	Provided North Brunswick Field Office with a copy of Discovery's October 31, 2007 submission.
11/12/2007	Response to FDA Request for Additional Information	Provided draft labeling for resubmission dated October 31, 2007 to the Electronic Document Room.
11/15/2007	FDA Facsimile	FDA's acknowledgment of Discovery's October 31, 2007 resubmission, which is considered a complete, class 2 response to the March 31, 2006 Approvable Letter.
11/15/2007	FDA Letter	FDA's acknowledgment of Discovery's October 31, 2007 resubmission, which is considered a complete, class 2 response to the March 31, 2006 Approvable Letter. US Mail-date received 11/28/2007.
11/26/2007	General Correspondence	Provided additional desk copies of Discovery's resubmission dated October 31, 2007 (Module 1 & Response Items) to Regulatory Project Manager, at the FDA.
11/29/2007	FDA Request for Additional Information	FDA request for additional CMC information to continue the review of Discovery's 10/31/07 submission (electronic version- date received 11/29/2007 and US mail version- date received 12/05/2007).
11/30/2007	General Correspondence Response to FDA Observations from Form FDA 483 Dated September 24, 2007	Provided DSCO's response to the 483 dated 9/24/2007 to the Philadelphia District Office. Response includes 23 Exhibits.
12/5/2007	Response to FDA 483 Dated 9/24/07	Provided the Agency with a copy of the text portion of the submission package dated 11/30/07 to the FDA's Philadelphia District Office regarding the 483 dated 9/24/07.
12/7/2007	General Correspondence Response to FDA Request for Additional Information	Provided a written response to Agency's 11/29/2007 information request letter regarding closure dates of Totowa and date available for inspection, dates for stability data, and format of stability data.
12/10/2007	General Correspondence	Provided one additional desk copy of Discovery's resubmission dated 10/31/2007 (Module 1 & Response Items) to Regulatory Project Manager at the FDA.

Date	*Type of Submission*	*Description of Submission*
12/20/2007	General Correspondence Response to FDA Request for Additional Information	Provided an update to Discovery's 12/7/2007 written response to the Agency's 11/29/2007 information request letter.
12/21/2007	General Correspondence Response to FDA Request for Additional Information	Provided a written response to Agency's 11/29/2007 information request letter.
1/4/2008	General Correspondence	Provided a copy of the draft labeling that was included in Discovery's 10/31/2007 in SPL format.
1/4/2008	General Correspondence	Provided NJ District Field Office with a copy of Discovery's 12/21/2007 submission to the Division regarding the Agency's 11/29/2007 information request letter.
1/8/2008	General Correspondence (E-mail) Response to FDA Request for Additional Information	Provided response to LA District Field Office's 1/8/08 request regarding testing facility. Information included: background information on SURFAXIN and all applicable sections from Discovery's 10/31/07 response which refer to testing.
1/11/2008	FDA Letter Request for Additional Information	FDA Request for an updated list of all sites involved in the manufacturing and testing of the drug product and drug substances, including activities performed, and contact information. For sites no longer involved date of last involvement and whether or not the site should remain active as an alternate site.
1/18/2008	General Correspondence Response to FDA Request for Additional Information	Provided a written response to the Agency's 1/11/2008 request. Items included: updated list of all sites currently involved in the manufacturing and testing of the drug product and drug substances and replacement pages for sections 3.2.S.2.1 and 3.2.P.3.1 (Manufacturers) of Module 3 to reflect the updated list of manufacturing and testing sites.
1/21/2008	General Correspondence Response to FDA Request for Additional Information	Provided response to Wilmington DE office 1/18/2008 request regarding testing facility. Provided information via e-mail. Information included: Sections 3.2.P.3.1, 3.2.P.2.5., 3.2.P.8.2, and 3.2.P.5.1 from October 31, 2007 complete response.
1/25/2008	General Correspondence Response to FDA Request for Additional Information	Provided the NJ District Office with a copy of DSCO's 1/18/2008 response to the Agency's 1/11/2008 information request letter. Items included: Updated list of all sites currently involved in the manufacturing and testing of the drug product and drug substances and replacement pages for sections 3.2.S.2.1 and 3.2.P.3.1 (Manufacturers) of Module 3 to reflect the updated list of manufacturing and testing sites.
2/29/2008	General Correspondence Response to FDA Request for Additional Information	Provided the Agency with stability data on Surfaxin new process validation lots.
3/3/2008	General Correspondence Response to FDA Request for Additional Information	Provided the NJ District Office with a coy of DSCO's 2/29/08 submission that included stability data on Surfaxin new process validation lots.
4/7/2008	General Correspondence Response to FDA Observations from Form FDA 483 Dated 3/25/2008	Provided the Parsippay, NJ District Office with Discovery's response to the Form FDA 483 Dated March 25, 2008. (Totowa SOPs provided as attachments).
4/10/2008	General Correspondence Response to FDA Observations from Form FDA 483 Dated 3/25/2008	Provided the Division with a copy of Discovery's 4/7/2008 response to the Parsippay, NJ District Office regarding the Form FDA 483 Dated 3/25/2008. (Totowa SOPs provided as attachments).
4/14/2008	FDA Facsimile (Sent via Email)	FDA Comments regarding DSCO's draft labeling submitted in DSCO's resubmission dated 10/31/2007. (Submitted to Electronic Document Room 11/12/2007).

Date	*Type of Submission*	*Description of Submission*
4/21/2008	General Correspondence	DSCO Response to FDA Labeling Comments Dated 4/14/2008. Attachments include draft PI.
4/25/2008	FDA Facsimile (Sent via Email)	FDA Comments regarding DSCO's draft labeling (PI) submitted 4/21/2008.
4/29/2008	General Correspondence Response to FDA Request for Additional Information	Provided the Agency with DSCO's revised draft package insert dated 4/28/08 in response to the Agency's 4/25/08 fax.
4/29/2008	General Correspondence Response to FDA Request for Additional Information	Provided the Agency with DSCO's revised draft vial and carton labels in response to the Agency's 4/14/08 comments.
4/29/2008	General Correspondence Response to FDA Request for Additional Information	Provided the Agency with information regarding activities/responsibilities that were transferred from Laureate Princeton, NJ to Discovery.
5/1/2008	FDA Letter	FDA's Approvable Letter (18 CMC Comments and 2 Draft Labeling Comments) (electronic version dated 5/1/2008, US mail date received 05/06/2008).
5/5/2008	General Correspondence	Notification of Intent to File Amendment to the Approvable Letter Dated 5/1/2008.
5/14/2008	General Correspondence	End of Review Meeting Request/Package- Regarding Approvable Letter Dated 5/1/2008.
5/20/2008	General Correspondence	Desk Copies for June 18, 2008 End-of Review Meeting
5/28/2008	General Correspondence	FDA confirmation of End- of-Review meeting scheduled for 06/18/2008.
6/3/2008	General Correspondence	Provided the Agency with 12 desk copies of June 3, 2008 Meeting Submission (Supplement to the May 14, 2008 End of Review Meeting Package).
6/3/2008	General Correspondence	Provided the Agency with 3 copies of Meeting Submission-Supplement to the May 14, 2008 End of Review Meeting Package.
6/17/2008	FDA Facsimile (Sent via Email)	FDA comments regarding DSCO's questions submitted in the May 14, 2008 Meeting Package Submission
7/14/2008	FDA Meeting Minutes	FDA Meeting Minutes from June 18, 2008 Teleconference
10/17/2008	Resubmission of NDA Application	Provided Discovery's complete response to the Agency's May 1, 2008 Approvable Letter (Total of 7 volumes). Response consisted of the following: Response Items (1 volumes), Module 1 (1 volume), Module 3 (2 volumes), and Method Validation Package (3 volumes).
10/17/2008	General Correspondence	Stamped Received Cover Letters for Resubmission dated 17October2008 from the Division, Division EDR (includes CD ROM) and NJ Field Office.
11/7/2008	FDA Letter	FDA response to the 10/17/2008 resubmission. The response is considered a Class 2 response (electronic version). Letter received via US mail received 11/20/2008.
11/25/2008	General Correspondence	Provided 4 additional copies of Resubmission dated 10/17/2008- Response Items (1 Vol.), Mod 1 (Vol 1) and Mod 3 (Vol 1 and 2).
2/12/2009	General Correspondence	Tightening of Surfaxin Drug Product Acceptance Criteria (Revised Proposed Limits for Lipid-Related Impurities DG2 and DPPA)
2/17/2009	General Correspondence	Field Copy of Minor Amendment sent on 02/12/2009 regarding; Tightening of Surfaxin Drug Product Acceptance Criteria.
3/12/2009	General Correspondence	Response to March 4, 2009 Teleconference.
3/13/2009	Response to FDA Request for Additional Information	1 Desk Copy of April 7,2008 submission to the NJ District Office
4/17/2009	FDA Complete Response Letter	FDA Complete Response Letter.

Date	*Type of Submission*	*Description of Submission*
4/24/2009	General Correspondence End-of-Review Meeting Request/Package	End-of-Review Meeting Request/Package to discuss FDA's comments regarding the in-vivo BAT and Discovery's reliance on literature from the April 17, 2009 Complete Response Letter.
5/11/2009	End-of-Review Meeting Request Grant Letter	FDA's End-of-Review meeting request grant letter.
5/19/2009	General Correspondence Supplement to April 24, 2009 End-of-Review Meeting Request/Package	Supplement to April 24, 2009 End-of-Review Meeting Request/Package.
5/21/2009	General Correspondence	Request to expunge March 27, 2009 letter.
6/1/2009	General Correspondence	FDA Responses to Questions in Briefing Package (April 24, 2009 original request and May 19, 2009 supplement) for June 2, 2009 meeting.
6/16/2009	General Correspondence FDA Meeting Minutes	FDA Meeting Minutes from June 2, 2009 Meeting (Received via US Mail on 6/23/2009)
6/26/2009	General Correspondence	Proposed revisions to the FDA's meeting minutes dated June 16, 2009 from the June 2, 2009 meeting.
8/5/2009	General Correspondence Type C Meeting Request/Briefing Package	Meeting Request/Package to discuss the design of a limited clinical trial.
9/2/2009	General Correspondence FDA Meeting Grant Letter	FDA Meeting Grant Letter in response to August 5, 2009 meeting request. Type C Meeting Date September 29, 2009 (Received via e-mail 9/2 and US Mail 9/9)
9/2/2009	General Correspondence Additional Copies of August 5, 2009 Type C Meeting Request/Package	Provided 3 additional copies of August 5, 2009 Type C Meeting Request/Package.
9/11/2009	General Correspondence Updated Discovery Meeting Attendee List	Provided updated Discovery attendee list for September 29, 2009 teleconference.

Exhibit J

PTO/SB/80 (11-08)
Approved for use through 11/30/2011. OMB 0651-0035
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

POWER OF ATTORNEY TO PROSECUTE APPLICATIONS BEFORE THE USPTO

I hereby revoke all previous powers of attorney given in the application identified in the attached statement under 37 CFR 3.73(b).

I hereby appoint:

☑ Practitioners associated with the Customer Number: 37509

OR

☐ Practitioner(s) named below (if more than ten patent practitioners are to be named, then a customer number must be used):

Name	Registration Number	Name	Registration Number

as attorney(s) or agent(s) to represent the undersigned before the United States Patent and Trademark Office (USPTO) in connection with any and all patent applications assigned only to the undersigned according to the USPTO assignment records or assignment documents attached to this form in accordance with 37 CFR 3.73(b).

Please change the correspondence address for the application identified in the attached statement under 37 CFR 3.73(b) to:

☑ The address associated with Customer Number: 37509

OR

☐ Firm or Individual Name			
Address			
City		State	Zip
Country			
Telephone		Email	

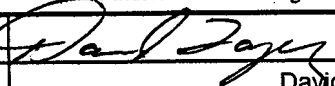
Assignee Name and Address:

Agent of Assignee: Discovery Laboratories, Inc., 2600 Kelly Road, Suite 100, Warrington, PA 18976-3622;
Assignee: The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037

A copy of this form, together with a statement under 37 CFR 3.73(b) (Form PTO/SB/96 or equivalent) is required to be filed in each application in which this form is used. The statement under 37 CFR 3.73(b) may be completed by one of the practitioners appointed in this form if the appointed practitioner is authorized to act on behalf of the assignee, and must identify the application in which this Power of Attorney is to be filed.

SIGNATURE of Assignee of Record

The individual whose signature and title is supplied below is authorized to act on behalf of the assignee

Signature		Date	9/16/09
Name	David L. Lopez, Esq.	Telephone	
Title	Executive Vice President, General Counsel of Discovery Laboratories, Inc.		

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Patentee:	Charles G. COCHRANE, *et al.*	Docket No:	TSRI-147.2CO
Patent No.:	5,789,381	Group Art Unit:	1652
Issued:	Aug. 4, 1998	Confirmation No.:	4428
For:	**PULMONARY SURFACTANT PROTEINS AND RELATED POLYPEPTIDES**	Examiner:	Patrick R DELANEY

COMBINED POWER OF ATTORNEY BY AGENT OF ASSIGNEE AND STATEMENTS UNDER 37 CFR §§ 3.73(B) AND 3.71

Discovery Laboratories, Inc. (hereinafter "Discovery"), a Delaware corporation, states that it is the Agent of the assignee of the entire right, title and interest in the above-captioned patent by virtue of an Appointment of Agency from the assignee, The Scripps Research Institute (hereinafter "Scripps"), attached hereto as Exhibit A. Pursuant to the Appointment of Agency, Discovery has authority to transact all business in the United States Patent and Trademark Office in connection with U.S. Patent No. 5,789,381, including subsmitting applications for patent term extension under 35 U.S.C. §156.

Scripps is the assignee of the entire right, title and interest in the above-captioned patent application by virtue of an assignment from the inventor(s) of the above-captioned application recorded in the United States Patent and Trademark Office at Reel 015215, Frame 0518 on Aug. 4, 1998.

Discovery hereby revokes all previous powers of attorney and appoints **Ann M. Caviani Pease**, Registration No. 42,067, and the **Dechert LLP** attorneys and agents associated with **Customer Number 37509** as its attorneys with full power of substitution and revocation, to transact all business in the Patent and Trademark Office connected with the captioned patent, said appointment being to the exclusion of the inventor(s) and his/her attorney(s) in accordance with the provisions of 37 CFR § 3.71; provided that if any one of said attorneys or agents ceases to be affiliated with the law firm of Dechert LLP as partner, employee or of counsel, such attorney or agent's appointment as attorney and all powers derived therefrom shall terminate on the date such attorney or agent ceases being so affiliated.

Please direct all correspondence address for the above-identified application to:

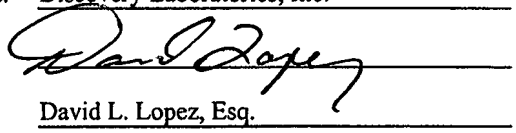
Customer Number **37509**
Firm or Individual Name:

Address: Dechert LLP, P.O. Box 390460, Mountain View, CA 94039-0460
Telephone: 650-813-4800

The undersigned, whose title is supplied below, is authorized to act on behalf of the Agent.

Assignee Agent: Discovery Laboratories, Inc.

Date: Sept. 16, 2009

Signed: 

Print Name: David L. Lopez, Esq.

Print Title: Executive Vice President, General Counsel